(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 October 2007 (04.10.2007)

PCT

(10) International Publication Number
WO 2007/112357 A2

(51) International Patent Classification:
A61K 38/13 (2006.01)

(21) International Application Number: PCT/US2007/064942

(22) International Filing Date: 26 March 2007 (26.03.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
11/391,020 28 March 2006 (28.03.2006) US

(71) Applicant (*for all designated States except US*): **AMR TECHNOLOGY, INC.** [US/US]; 5429 Main Street, P.O. Box 2587, Manchester Center, VT 05255-2587 (US).

(72) Inventor: **MOLINO, Bruce, F.**; 14 Cygnet Circle, Slingerlands, NY 12159 (US).

(74) Agents: **CHOI, Alice, Y.** et al.; Nixon Peabody LLP, Clinton Square, P.O. Box 31051, Rochester, NY 14603-1051 (US).

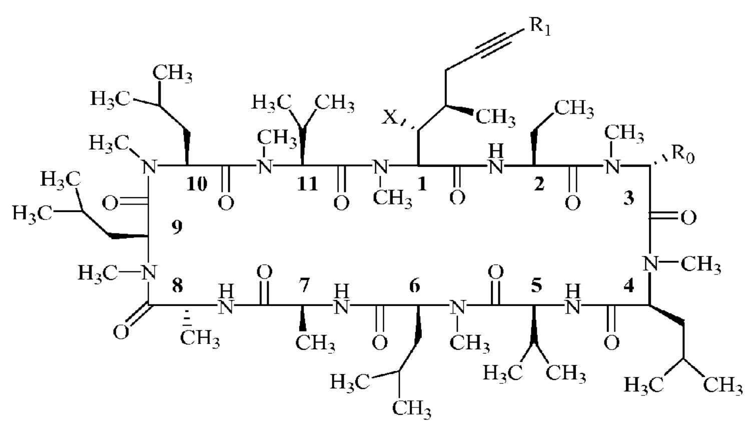
(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: USE OF CYCLOSPORIN ALKYNE ANALOGUES FOR PREVENTING OR TREATING VIRAL-INDUCED DISORDERS



(I)

(57) Abstract: The present invention relates to methods of preventing or treating a mammal with a viral-induced disorder. The method involves administering to the mammal a therapeutically effective amount of a compound represented by Formula (I), as shown below: (Formula I) or a pharmaceutically acceptable salt thereof, with X, R_0, and R_1 defined herein, under conditions effective to prevent or treat the viral-induced disorder.

WO 2007/112357 A2

USE OF CYCLOSPORIN ALKYNE ANALOGUES FOR PREVENTING OR TREATING VIRAL-INDUCED DISORDERS

[0001] This application claims the benefit of U.S. Patent Application Serial No. 11/391,020, filed March 28, 2006, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention discloses novel cyclosporin alkyne analogues and their utility as pharmaceutical agents for prevention and treatment of viral-induced diseases. Methods for preparation of such compounds are also disclosed.

BACKGROUND OF THE INVENTION

[0003] Cyclosporin A (CsA), a neutral cyclic undecapeptide isolated from the fungus *Tolypocladium inflatum* and currently marketed as Neoral® and Sandimmune® (Novartis, Basel, Switzerland), has been widely used for the prevention of organ transplant rejection. The molecular basis for the immunosuppressant activity of cyclosporin A and cyclosporin analogues begins with the passive diffusion of the cyclosporin (Cs) molecule into the cell, followed by binding to its intracellular receptor, cyclophilin A (CypA). CypA belongs to a family of proteins that catalyze *cis-trans* peptidyl-prolyl isomerization, i.e., PPIase, a rate-limiting step in protein folding. CsA and other cyclosporin analogues bind to the active site of CypA. However, immunosuppression is not believed to be due to the inhibition of CypA PPIase activity. The target of the CsA-CypA complex is a Ca^{2+}-calmodulin-dependent serine-threonine-specific protein phosphatase, calcineurin. In T-cells responding to antigen presentation, an increase in intracellular Ca^{2+} activates calcineurin, which subsequently dephosphorylates the transcription factor called the nuclear factor of activated T-cells ("NFAT"). Dephosphorylated NFAT undergoes a molecular change, e.g., homodimerization that allows it to cross into the nucleus, and promotes the expression of T-cell activation genes. CsA and other immunosuppressive cyclosporin derivatives inhibit calcineurin which results in the inhibition of expression of cytokine genes, e.g., interleukin-2 (IL-2) that promotes T-cell activation and proliferation, i.e., immunosuppressive activity.

Human Immunodeficiency Viruses and Cyclosporin A or Non-Immunosuppressive Cyclosporins

[0004] Human immunodeficiency viruses ("HIVs") are lentiviruses, a family of mammalian retroviruses evolved to establish chronic persistent infection with gradual onset of clinical symptoms. There are two major families of HIV. Most of the epidemic involves HIV-1; HIV-2 is a close relative whose distribution is concentrated in western Africa.

[0005] Human cyclophilins A and B have been identified as cellular proteins which bind specifically to HIV-1 Gag polyprotein, $p555^{gag}$. Gag proteins play a major role in several steps of the virus life cycle, including the assembly and release of virions (Willis et al., "Form, Function, and Use of Retroviral Gag Proteins," *AIDS* 5:639-654 (1991)). A cleavage product of the Gag polyprotein, the capsid protein, has been shown to bind specifically to cyclophilin A. Cyclophilin A is functionally associated with the HIV-1 virions through interaction with the Gag polyprotein. This interaction between cyclophilin A and Gag proteins is inhibited by the immunosuppressive drug, cyclosporin A (Thali et al., "Functional Association of Cyclophilin A With HIV-1 Virions," *Nature* 372:363-365 (1994)).

[0006] Cyclosporin A has demonstrated *in vitro* antiviral activity against HIV-1 (Karpas et al., "Inhibition of Human Immunodeficiency Virus and Growth of Infected T-cells by the Immunosuppressive Drugs Cyclosporin A and FK 506," *Proc. Natl. Acad. Sci. USA* 89:8351-8355 (1992)); however, initial *in vivo* studies in which cyclosporin A was administered as a monotherapy in HIV-infected patients at advanced stages of disease did not show a beneficial effect from the treatment (Levy et al., "Long-Term Follow-Up of HIV Positive Asymptomatic Patients Having Received Cyclosporin A," *Adv. Ex. Med. Biol.* 374:229-234 (1995)). U.S. Patent No. 4,814,323 to Andrieu et al. reported that administration of cyclosporins may be used for the prevention of AIDS in patients infected with the virus before the appearance of the AIDS symptoms, that is patients with no symptoms or patients with AIDS related complex.

[0007] Highly active antiretroviral therapy ("HAART") has dramatically decreased the HIV-related morbidity and mortality rates among HIV-infected patients and the transmission of HIV from mother to child by efficiently suppressing viral

replication (Palella et al., "Declining Morbidity and Mortality Among Patients With Advanced Human Immunodeficiency Virus Infection," *N. Eng. J. Med.* 338:853-860 (1998)). Limitations of HAART have become better understood. Thus, the virus can be suppressed to undetectable levels but not eradicated. In addition, there is an ever-growing list of side effects, the eventual development of resistance, and the cost and complexity of HAART regimens that must be contended with.

[0008] HAART covers a broad range of antiretroviral agents that include nucleoside reverse transcriptase inhibitors ("NRTI"), nonnucleoside reverse transcriptase inhibitors ("NNRTI"), HIV protease inhibitors, and fusion inhibitors. Specific examples of antiviral agents from each of these families include: Zidovudine, Didanosine, Stavudine, and Lamivudine from the NRTI antiviral class; Nevirapine, Efavirenz, and Delavirdine from the NNRTI antiviral class; Saquinovir, Indinavir, and Ritonavir from the HIV protease inhibitor class; and Enfuvirtide from the fusion inhibitor antiviral class.

[0009] From an immunological standpoint, the introduction of HAART allows for only a partial immune reconstitution. Indeed, *ex vivo* measures of immune function do not generally normalize and, most importantly, HIV-specific T cell responses remain almost invariably impaired. Though several variables have been identified that correlate with the degree of immune reconstitution during HAART, the actual underlying mechanism(s) responsible for such an incomplete immune reconstitution are still poorly understood and likely reflect the severe HIV-driven perturbations in T cell dynamics and homeostasis and the interaction between host and viral factors (Douek, "Disrupting T-Cell Homeostasis: How HIV-1 Infection Causes Disease," *AIDS Rev.* 5:172-177 (2003)).

[0010] A strategy aimed at the broadest immune reconstitution, possibly overcoming the limitations of HAART, consists in the adjuvant use of immunomodulants. By combining cyclosporin A with HAART, the goal is to contain the immune activation, either virus-specific or owing to non-specific "by-stander" activation. Results from pilot studies in HIV-infected patients has shown that the rapid shutdown of T-cell activation induced by cyclosporin A has produced a more rapid and stable increase in CD4+ T-cells and a significant long-term increase in IFN-γ secreting CD4+ and CD4+CCR7- T-cells, establishing a more favorable immunological set-point (Bandera et al., "Immunomodulants in HIV Infection,"

Expert Opin. Ther. Patents 15(9):1115-1131 (2005)). Determination of the long-term efficacy must be assessed in order to understand if this approach truly has value.

[0011] SDZ NIM 811 is a cyclosporin analogue that is completely devoid of immunosuppressive activity but exhibits potent and selective anti-HIV-1 activity (Mlynar et al., "The Non-Immunosuppressive Cyclosporin A Analogue SDZ NIM 811 Inhibits Cyclophilin A Incorporation Into Virions and Virus Replication in Human Immunodeficiency Virus Type-1-Infected Primary and Growth-Arrested Cells," *J. General Virology* 78:825-835 (1997)). SDZ NIM 811 does not prevent the activation of CD4+ T-cell activation as cyclosporin A does. In a manner similar to cyclosporin A, it is proposed that SDZ NIM 811 interferes with the HIV-1 Gag-cyclophilin A interaction to effect its antiviral activity.

[0012] SDZ NIM 811 does not inhibit calcineurin and possesses none of the immunosuppressive actiivity of cyclosporin A. The potent inhibition of calcineurin by cyclosporin, in addition to being responsible for the potent immunosuppressive activity of cyclosporin A, is also believed to be the cause of the toxicity and the narrow therapeutic index of this drug. Separation of immunosuppressive and antiviral activity could lead to novel antiviral cyclosporins with fewer side effects and improved therapeutic index. Elucidation of structure activity relationships for cyclosporins permits the design of non-immunosuppressive cyclosporin derivatives that retain potent (cyclophilin A) PPIase activity to achieve this goal (Bartz et al., "Inhibition of Human Immunodeficiency Virus Replication by Non-Immunosuppressive Analogs of Cyclosporin A," *Proc. Natl. Acad. Sci. USA* 92:5381-5385 (1995)). European Patent No. 484 281, U.S. Patent No. 5,767,069, U.S. Patent No. 5,948,884, and French Patent Nos. 2,757,520, 2,757,521, and 2,757,522 disclose non-immunosuppressive cyclosporins with antiviral activity.

Hepatitis C Virus and Cyclosporin A

[0013] Recently, cyclosporin A, the most widely prescribed immunosuppressive drug, was reported to be clinically effective against hepatitis C viral (HCV) infection (Nakagawa et al., "Specific Inhibition of Hepatitis C Virus Replication by Cyclosporin A," *Biochem. Biophys. Res. Commun.* 313:42-47 (2004)). The authors of the Nakagawa et al. paper state that certain chaperone activities, such

as those of cyclophilins, may be crucial for the processing and maturation of the viral proteins and for viral replication.

[0014] A subsequent controlled clinical trial showed that a combination of cyclosporin A with interferon α2b is more effective than interferon monotherapy, especially in patients with high viral loads (Inoue et al., "Combined Interferon α2b and Cyclosporin A in the Treatment of Chronic Hepatitis C: Controlled Trial," *J. Gastroenterol.* 38:567-572 (2003)).

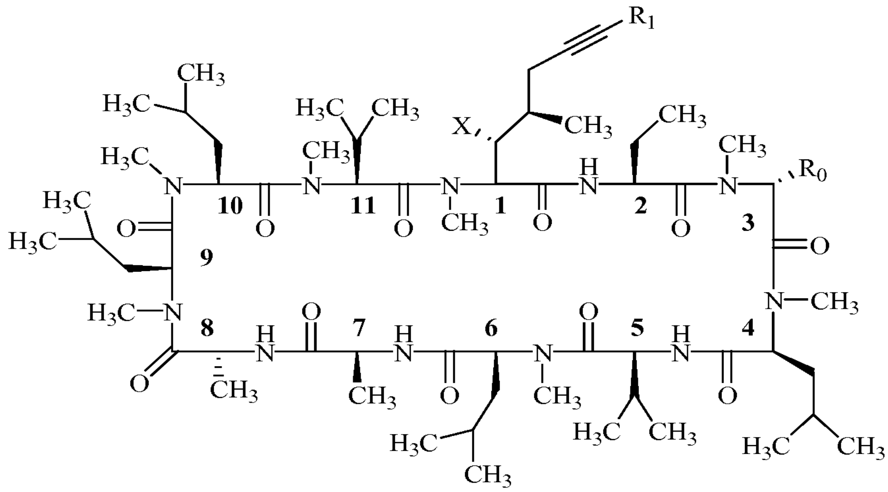
[0015] PCT International Patent Publication No. WO 2006/005610 recently described the use of a combination of cyclosporin A and pegylated interferon for treating hepatitis C viral infection. In addition, PCT International Patent Publication No. WO 2005/021028 relates to the use of non-immunosuppressive cyclosporins for treatment of HCV disorders. Also, Paeshuyse et al., "Potent and Selective Inhibition of Hepatitis C Virus Replication by the Non-Immunosuppressive Cyclosporin Analogue DEBIO-025," *Antiviral Research* 65(3):A41 (2005) recently published results for a non-immunosuppressive cyclosporin analogue, DEBIO-025, that exhibited potent and selective inhibition of hepatitis C virus replication. Notably, the cyclosporin derivative DEBIO-025 is also effective for the treatment of HIV-1 (Rosenwirth et al., "Debio-025, A Novel Non-Immunosuppressive Cyclosporine Analog with Potent Anti-Human Immunodeficiency Virus Type 1 Activity: Pharmacological Properties and Mode of Action," *Antiviral Research* 65(3):A42-A43 (2005)). Debio-025 does possess potent binding affinity for cyclophilin A.

[0016] There is still a large need for novel cyclosporin analogues that have therapeutic utility in the treatment of viral-induced diseases.

[0017] The present invention is directed to achieving these objectives.

SUMMARY OF THE INVENTION

[0018] The present invention relates to a method of preventing or treating a mammal with a viral-induced disorder. The method involves administering to the mammal a therapeutically effective amount of a compound having the following formula:



Formula I

where:

X is OH or OAc;

R_0 is H, CH_2OH, or CH_2OR_2;

R_1 is selected from the group consisting of:
- hydrogen;
- halogen;
- C_2-C_6 saturated or unsaturated, straight or branched carbon chain;
- C_2-C_6 saturated or unsaturated, straight or branched carbon chain containing substitution or substitutions selected from the group consisting of deuterium, halogen, nitrogen, sulfur, and silicon atom or atoms;
- C_2-C_6 saturated or unsaturated, straight or branched carbon chain containing a function group or function groups selected from the group consisting of alcohol, ether, aldehyde, ketone, carboxylic ester, and amide;
- C_2-C_4 saturated or unsaturated, straight or branched carbon chain containing an aryl or a heteroaryl;
- C_3-C_6-substituted and unsubstituted cycloalkyl;
- substituted and unsubstituted aryl;
- substituted and unsubstituted heteroaryl;
- $-CH_2OH$;
- -CHO;
- $-CH=N-OR_3$; and
- $-CH=N-NR_3R_4$;

R_2 is selected from the group consisting of:
- alkanoyl;
- alkenoyl;
- alkynoyl;
- aryloyl;
- arylalkanoyl;

alkylaminocarbonyl;
arylaminocarbonyl;
arylalkylaminocarbonyl;
alkyloxycarbonyl;
aryloxycarbonyl; and
arylalkyloxycarbonyl;

R_3 or R_4 are the same or different and independently selected from the group consisting of:

hydrogen;
C_1-C_6 saturated straight or branched carbon chain;
C_3-C_6 unsaturated straight or branched carbon chain;
C_3-C_6-substituted and unsubstituted cycloalkyl;
C_1-C_4 carbon chain containing an aryl or heteroaryl;
substituted and unsubstituted aryl;
substituted and unsubstituted heteroaryl;
alkanoyl;
alkenoyl;
alkynoyl;
aryloyl;
arylalkanoyl;
alkylaminocarbonyl;
arylaminocarbonyl;
arylalkylaminocarbonyl;
alkyloxycarbonyl;
aryloxycarbonyl; and
arylalkyloxycarbonyl; and

R_3 together with R_4 results in the formation of a cyclic moiety of C_2-C_6 optionally containing heteroatom or heteroatoms,

or a pharmaceutically acceptable salt thereof,

under conditions effective to prevent or treat the viral-induced disorder.

[0019] The present invention discloses chemically modified cyclosporin analogues containing a carbon-carbon triple bond on the side chain of the position one amino acid and optionally a substitution on the position three amino acid of cyclosporin A. In particular, the present invention discloses novel cyclosporin alkyne analogues containing a conjugated system of a carbon-carbon triple bond with an aryl, a carbon-carbon double bond, a carbon-nitrogen double bond, or another carbon-carbon triple bond.

[0020] The present invention discloses novel cyclosporin analogues which are effective as antiviral agents. The cyclosporin derivatives of the present invention used to treat viral infections may possess potent immunosuppressive activity (via

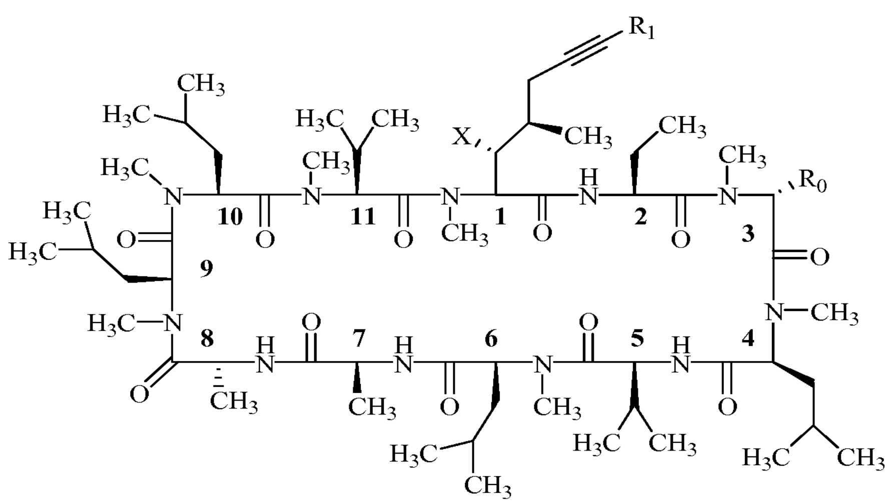
inhibition of calcineurin) or may be completely devoid of immunosuppressive activity (do not inhibit calcineurin). However, the mechanism that the immunosuppressive and non-immunosuppressive cyclosporin compounds share is their activity at cyclophilin A.

BRIEF DESCRIPTION OF THE DRAWING

[0021] Figure 1 depicts the results from a concanavalin A (ConA)-stimulated murine splenocyte assay, where the novel cyclosporin analogue compounds of the present invention (disclosed in Examples 25 and 10) are shown to possess enhanced or similar potency in immunosuppression, compared to cyclosporin A.

DETAILED DESCRIPTION OF THE INVENTION

[0022] The present invention relates to a method of preventing or treating a mammal with a viral-induced disorder. The method involves administering to the mammal a therapeutically effective amount of a compound having the following formula:



Formula I

where:

X is OH or OAc;

R_0 is H, CH_2OH, or CH_2OR_2;

R_1 is selected from the group consisting of:

hydrogen;

halogen;

C_2-C_6 saturated or unsaturated, straight or branched carbon chain;

C_2-C_6 saturated or unsaturated, straight or branched carbon chain containing substitution or substitutions selected from the group consisting of deuterium, halogen, nitrogen, sulfur, and silicon atom or atoms;

C_2-C_6 saturated or unsaturated, straight or branched carbon chain containing a function group or function groups selected from the group consisting of alcohol, ether, aldehyde, ketone, carboxylic ester, and amide;

C_2-C_4 saturated or unsaturated, straight or branched carbon chain containing an aryl or a heteroaryl;

C_3-C_6-substituted and unsubstituted cycloalkyl;

substituted and unsubstituted aryl;

substituted and unsubstituted heteroaryl;

-CH_2OH;

-CHO;

-CH=N-OR_3; and

-CH=N-NR_3R_4;

R_2 is selected from the group consisting of:

alkanoyl;

alkenoyl;

alkynoyl;

aryloyl;

arylalkanoyl;

alkylaminocarbonyl;

arylaminocarbonyl;

arylalkylaminocarbonyl;

alkyloxycarbonyl;

aryloxycarbonyl; and

arylalkyloxycarbonyl;

R_3 or R_4 are the same or different and independently selected from the group consisting of:

hydrogen;

C_1-C_6 saturated straight or branched carbon chain;

C_3-C_6 unsaturated straight or branched carbon chain;

C_3-C_6-substituted and unsubstituted cycloalkyl;

C_1-C_4 carbon chain containing an aryl or heteroaryl;

substituted and unsubstituted aryl;

substituted and unsubstituted heteroaryl;

alkanoyl;

alkenoyl;

alkynoyl;

aryloyl;

arylalkanoyl;

alkylaminocarbonyl;

arylaminocarbonyl;
arylalkylaminocarbonyl;
alkyloxycarbonyl;
aryloxycarbonyl; and
arylalkyloxycarbonyl; and

R_3 together with R_4 results in the formation of a cyclic moiety of C_2-C_6 optionally containing heteroatom or heteroatoms,

or a pharmaceutically acceptable salt thereof,

under conditions effective to prevent or treat the viral-induced disorder.

[0023] One embodiment of the present invention is the above compound of Formula I, where: X is OH or OAc; R_0 is H, CH_2OH, or CH_2OAc; and R_1 is H.

[0024] Another embodiment of the present invention is the above compound of Formula I, where: X is OH or OAc; R_0 is H, CH_2OH, or CH_2OAc; and R_1 is selected from the group consisting of F, Cl, Br, and I.

[0025] Another embodiment of the present invention is the above compound of Formula I, where: X is OH or OAc; R_0 is H, CH_2OH, or CH_2OAc; and R_1 is selected from the group consisting of $CH=CH_2$, $CH=CHCH_3$, $CH=CHCH_2CH_3$, $C(CH_3)=CH_2$, $CH=CD_2$, $CH=CHCD_3$, and $CH=CDCD_3$, and where the carbon-carbon double bond is a cis or a trans geometric isomer or a mixture of both cis and trans geometric isomers.

[0026] Another embodiment of the present invention is the above compound of Formula I, where: X is OH or OAc; R_0 is H, CH_2OH, or CH_2OAc; and R_1 is selected from the group consisting of $CH=CHF$, $CH=CHCl$, $CH=CHBr$, $CH=CHI$, $CH=CF_2$, and $CH=CCl_2$, and where the carbon-carbon double bond is a cis or a trans geometric isomer or a mixture of both cis and trans geometric isomers.

[0027] Another embodiment of the present invention is the above compound of Formula I, where: X is OH or OAc; R_0 is H, CH_2OH, or CH_2OAc; and R_1 is selected from the group consisting of $C\equiv CH$, $C\equiv CCH_3$, $C\equiv CCD_3$, $C\equiv CCH_2CH_3$, $C\equiv CCH_2CH_2CH_3$, and $C\equiv C$-cyclopropyl.

[0028] Another embodiment of the present invention is the above compound of Formula I, where: X is OH or OAc; R_0 is H, CH_2OH, or CH_2OAc; and R_1 is selected from the group consisting of $CH_2C\equiv CH$, $CH_2C\equiv CCH_3$, $CH_2C\equiv CCH_2CH_3$, $CH_2CH=CH_2$, $CH_2CH=CHCH_3$, and $CH_2CH=CHCH_2CH_3$ and where the carbon-

carbon double bond is a cis or a trans geometric isomer or a mixture of both cis and trans geometric isomers

[0029] Another embodiment of the present invention is the above compound of Formula I, where: X is OH or OAc; R_0 is H, CH_2OH, or CH_2OAc; and R_1 is selected from the group consisting of C≡C-C≡CH, C≡C-C≡CCH_3, C≡CCH=CH_2, C≡CCH=$CHCH_3$, CH=CHC≡CH, CH=CHC≡CCH_3, CH=CHCH=CH_2, and CH=CHCH=$CHCH_3$ and where the carbon-carbon double bond is a cis or a trans geometric isomer or a mixture of both cis and trans geometric isomers

[0030] Another embodiment of the present invention is the above compound of Formula I, where: X is OH or OAc; R_0 is H, CH_2OH, or CH_2OAc; and R_1 is cyclopropyl.

[0031] Another embodiment of the present invention is the above compound of Formula I, where: X is OH or OAc; R_0 is H, CH_2OH, or CH_2OAc; and R_1 is selected from the group consisting of CH_2OH, -CHO, CH(OH)CH_3, C(=O)CH_3, CH=N-OCH_3, CH=N-OCH_2CH_3, CH=N-$NHCH_3$, and CH=N-N$(CH_3)_2$.

[0032] Other embodiments of the present invention include the above compound of Formula I, where: X = OH or OAc; R_0 = H; and R_1 is selected from the group consisting of H, C_6H_5-, *p*-FC_6H_4-, *p*-$CH_3OC_6H_4$-, 2-thiophenyl, CH_2Ph, CH_2CH=CH_2, CH_2C≡CH, CH_2C≡$CHCH_3$, CH_2C≡CHSi$(CH_3)_3$, Br, CH_2Cl, CH=CH_2, CH=$CHCH_3$ (trans), CH=$CHCH_3$ (cis), CH=CHCl (trans), CH=CHCl (cis), CH=CHSi$(CH_3)_3$ (trans), C(CH_3)=CH_2, CH=CHPh, CH=$CHCO_2Et$ (cis), CH=C=CH_2, C≡CH, C≡CCH_3, C≡CCD_3, C≡CCH_2CH_3, C≡CC_4H_9, C≡CSi$(CH_3)_3$, C≡C-3-thiophene, C≡C-Ph, C≡CBr, C≡C-cyclopropyl, C≡C-cyclohexyl, C≡CCH_2OH, C≡CCH_2OCH_3, C≡$CCH_2SCH_2CH_3$, C≡$CCH_2N(CH_3)_2$, C≡CCH=CH_2, C≡CC(CH_3)=CH_2, C≡CCH=$CHCH_3$ (cis), C≡CCH=$CHCH_3$ (trans), CH=CHC≡CH, CH=CHC≡$CCH_2CH_2CH_3$, CH=CHC≡C-cyclopropyl, CH_2OH, CHO, C=N-OCH_3, and C=N-N$(CH_3)_2$.

[0033] Other embodiments of the present invention include the above compound of Formula I, where: X = OH or OAc; R_0 = CH_2OH or CH_2OAc; and R_1 is selected from the group consisting of H, CH=CH_2, CH=$CHCH_3$ (cis), CH=$CHCH_3$ (trans), and CH=CHCl (cis).

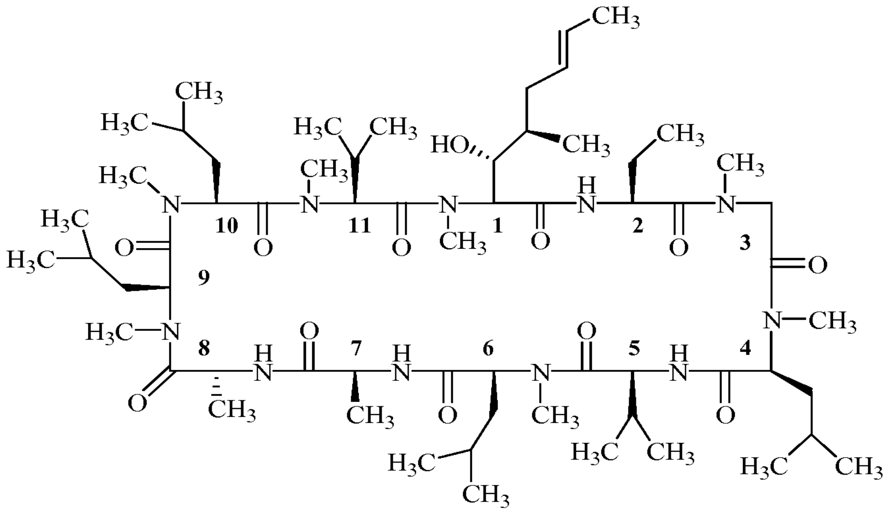
[0034] In particular, the present invention relates to novel cyclosporin analogues containing a carbon-carbon triple bond on the side chain of the position one

amino acid and optionally a substitution on the position three amino acid of cyclosporin A. More particularly, the present invention relates to novel cyclosporin alkyne analogues, in which the carbon-carbon triple bond conjugating with an aryl, or a heteroaryl, or a carbon-carbon double bond, or a carbon-nitrogen double bond, or another carbon-carbon triple bond is incorporated.

[0035] A carbon-carbon triple bond exists in many natural products (Gung et al., "Total Synthesis of (S)-(-)-(E)-15,16-Dihydrominquartynoic Acid: A Highly Potent Anticancer Agent," *J. Org. Chem.*, 69:3488-3492 (2004); Ito et al., "Cytotoxic Polyacetylenes from the Twigs of *Ochanostachys amentacea*," *J. Nat. Prod.*, 64:246-248 (2001), which are hereby incorporated by reference in their entirety). It is well known to use alkynes as pharmaceutical agents. However, only one cyclosporin alkyne, in which a carbon-carbon triple bond replaces the carbon-carbon double bond on the side-chain of the position one amino acid of cyclosporin A, is known in the literature. Unfortunately, this modification significantly reduces the immunosuppressive activity of cyclosporin A, where this known cyclosporin alkyne shows only 10% relative immunosuppressive activity, compared to cyclosporin A (Rich et al., "Synthesis, Conformation, and Immunosuppressive Activities of Three Analogues of Cyclosporin A Modified in the 1-Position," *J. Med. Chem.*, 33:999-1009 (1990), which is hereby incorporated by reference in its entirety). In contrast, the novel cyclosporin alkyne analogues of the present invention, which contain a conjugated system of a carbon-carbon triple bond and a carbon-carbon double bond or a carbon-carbon triple bond, possess enhanced immunosuppressive activity over cyclosporin A.

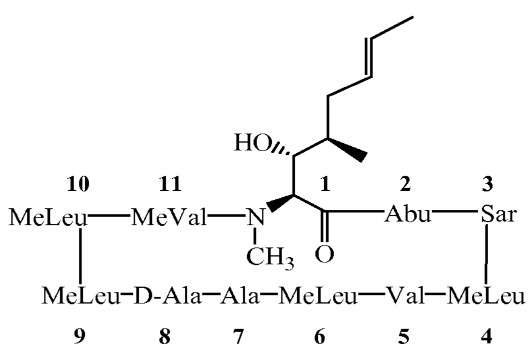
[0036] The present invention also discloses methods for preparing compounds represented by Formula I.

[0037] The starting material for the preparation of the compounds of the present invention is cyclosporin A. The structure of cyclosporin A, a cycloundecapeptide, and the position numbering for each amino acid in the ring is shown below:



Cyclosporin A (CsA)

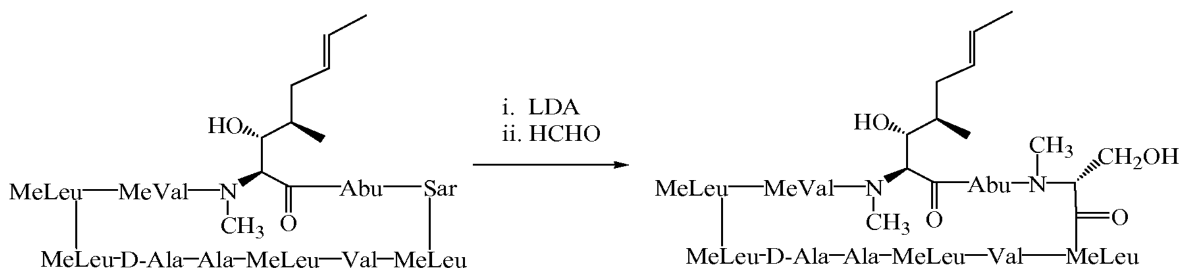
[0038] Cyclosporin A can also be represented by Formula IIa, as shown below:



Formula IIa

[0039] The novel cyclosporin analogues of the present invention are derived from cyclosporin A or cyclosporin diol (Formula IIb), a key intermediate prepared by modification on the position three amino acid of cyclosporin A. As shown in Scheme 1, the cyclosporin diol intermediate can be prepared by deprotonation of cyclosporin A with lithium diisopropylamide (LDA) followed by treatment with formaldehyde (Seebach et al, "Modification of Cyclosporin A: Generation of an Enolate at the Sarcosine Residue and Reaction with Electrophiles," *Helv. Chim. Acta*, 76:1564-1590 (1993), which is hereby incorporated by reference in its entirety).

Scheme 1

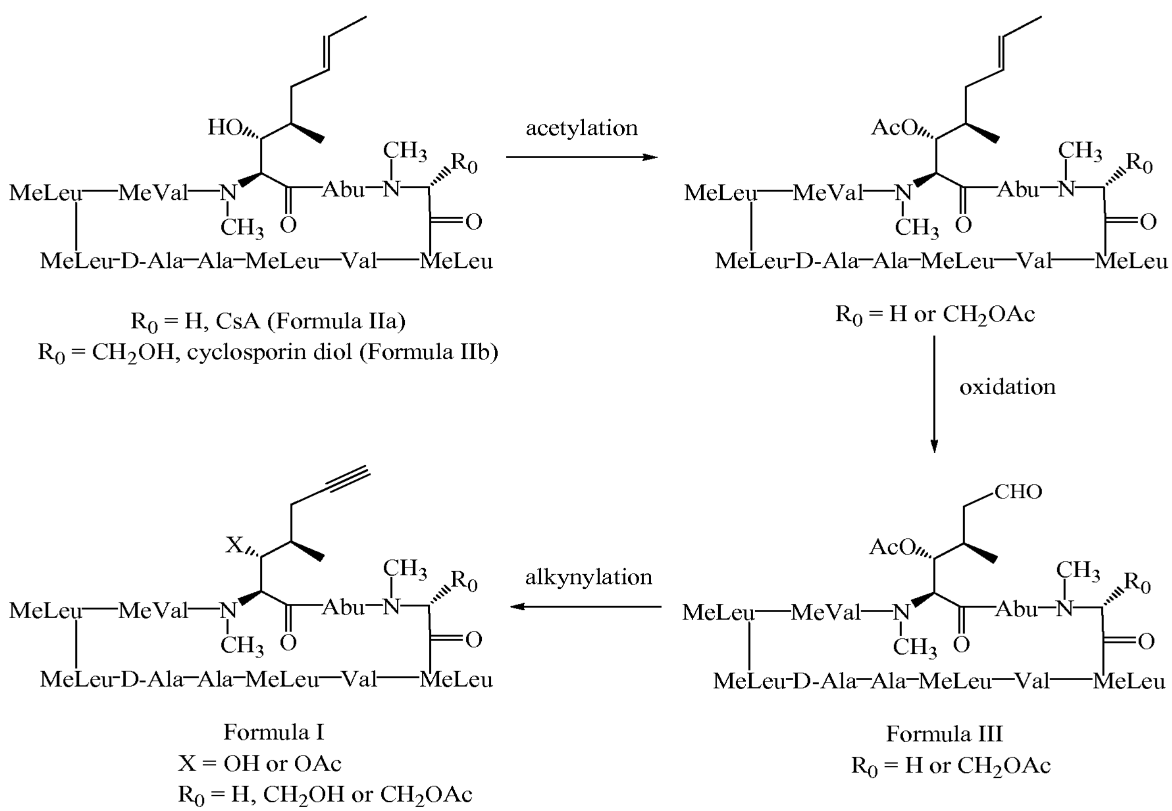


cyclosporin A
Formula IIa

cyclosporin diol
Formula IIb

[0040] According to one embodiment of the present invention, novel cyclosporin analogues can be prepared by replacing the carbon-carbon double bond on the side chain of the position one amino acid of cyclosporin A with a carbon-carbon triple bond. As depicted in Scheme 2, acetylation of cyclosporin A (Formula IIa) or the cyclosporin diol intermediate of Formula IIb with acetic anhydride, followed by oxidative cleavage of the double bond with ozone, generates the cyclosporin aldehyde of Formula III smoothly. Treatment of the cyclosporin aldehyde of Formula III with dimethyl (1-diazo-2-oxopropyl)phosphonate in the presence of potassium carbonate in methanol provides cyclosporin alkyne (Formula I, X = OH) in good yield (Müller et al, An Improved One-Pot Procedure for the Synthesis of Alkynes from Aldehydes," *Synlett*, 521-522 (1996), which is hereby incorporated by reference in its entirety). The acetyl protecting group can be removed under these reaction conditions to give the free alcohol directly.

Scheme 2

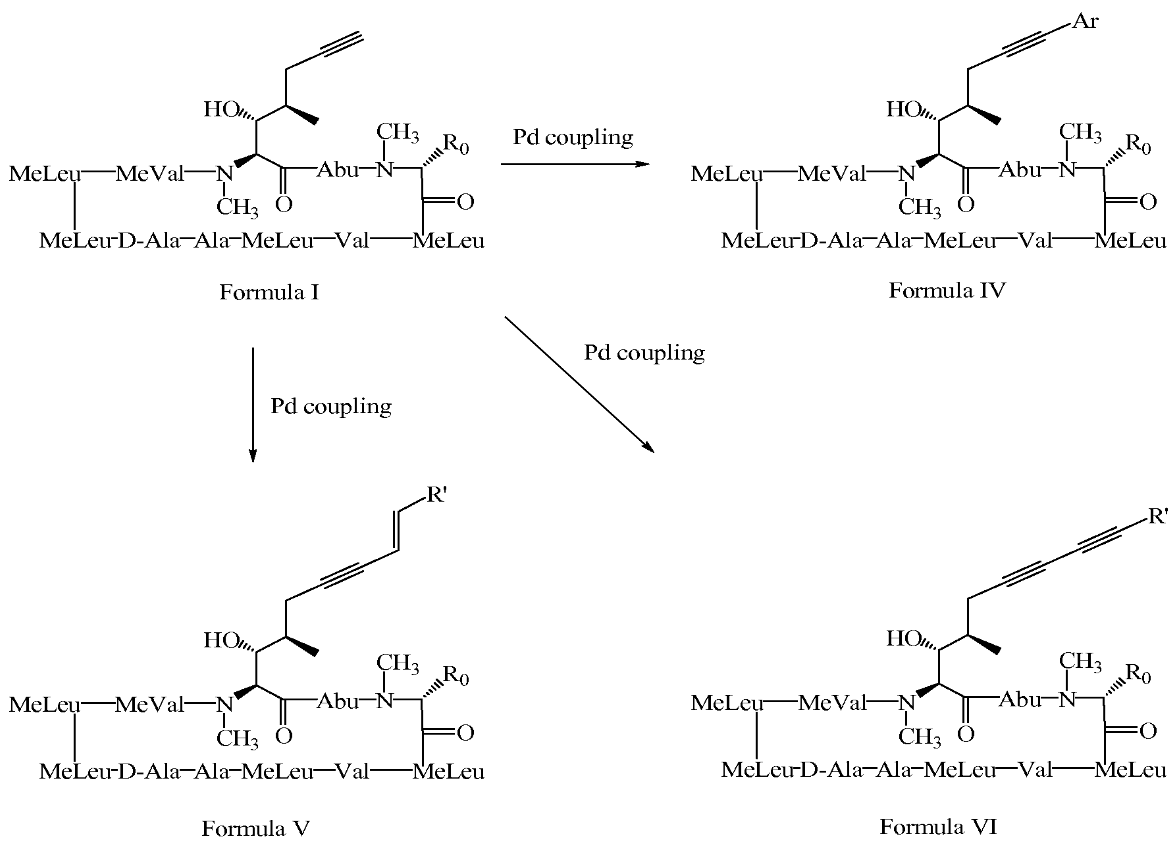


[0041] The cyclosporin aldehyde of Formula III can also be converted to the cyclosporin alkyne of Formula I (X = OH or OAc) via an alternate method (Scheme 2). Treatment of cyclosporin aldehyde with lithiotrimethylsilyldiazomethane affords the cyclosporin alkyne of Formula I (X = OH, R_0 = H or CH_2OH) in good yield (Ohira et al, "Generation of Alkylidenecarbenes by the Alkenation of Carbonyl Compounds with Lithiotrimethylsilyldiazomethane," *J. Chem. Soc. Chem. Commun.*, 721-722 (1992), which is hereby incorporated by reference in its entirety), while the reaction of cyclosporin aldehyde with lithiotrimethylsilyldiazomethane, followed by acidic workup (Ac_2O), provides the acetyl cyclosporin alkyne of Formula I (X = OAc, R_0 = H or CH_2OAc).

[0042] Using the above described cyclosporin alkyne (Formula I, X = OH) as a key intermediate, many novel cyclosporin alkyne derivatives can be prepared via palladium or nickel-mediated couplings. As shown in Scheme 3, Sonogashira coupling of cyclosporin alkyne (Formula I) with various aryl halides, heteroaryl halides, and vinyl halides provides novel cyclosporin arylated alkynes of Formula IV and cyclosporin yne-ene analogues of Formula V, respectively. Similarly, the

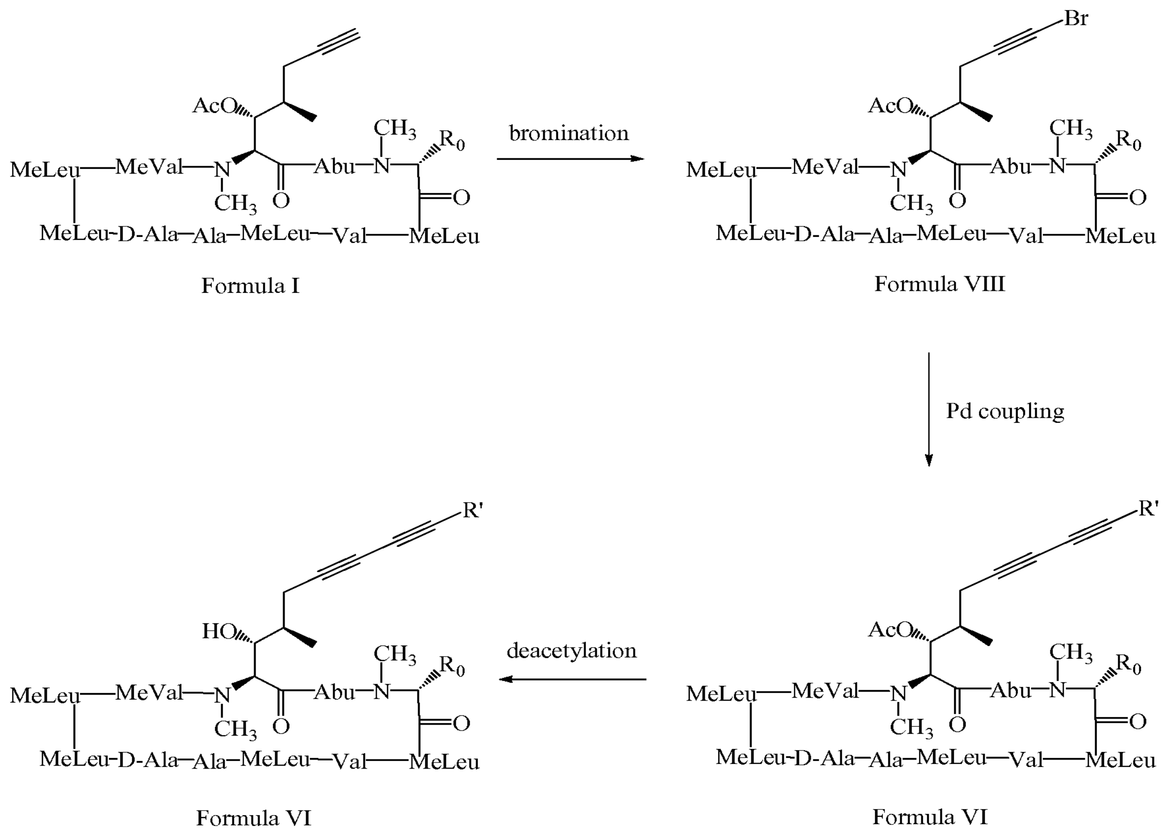
application of palladium-catalyzed coupling to the same key intermediate, cyclosporin alkyne (Formula I), with alkynyl halides leads to the preparation of novel cyclosporin diynes of Formula VI. Utilizing this method, a carbon-carbon triple bond could be introduced step by step to provide a conjugated system of multiple carbon-carbon triple bonds, such as triynes and tetraynes.

Scheme 3



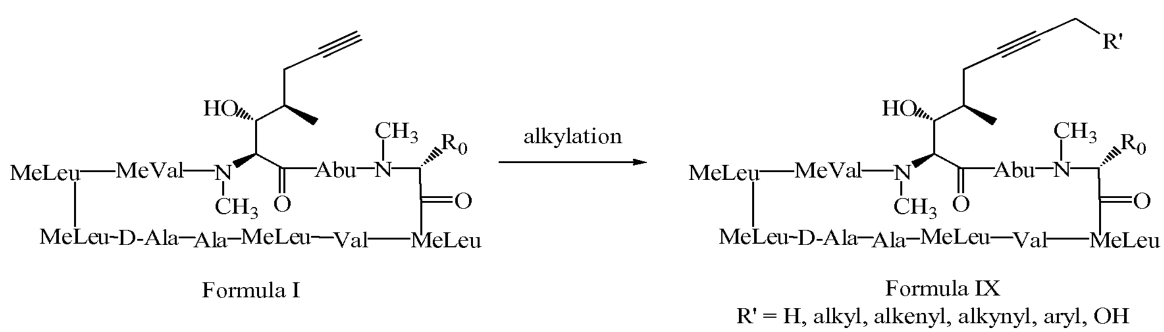
[0043] As shown in Scheme 4, the cyclosporin diynes of Formula VI can be prepared using an alternative approach. Bromination of cyclosporin alkyne (Formula I, X = OAc, R_1 = H) with *N*-bromosuccinimide in the presence of silver nitrate affords cyclosporin alkynyl bromide (Formula VIII). Using this method, other cyclosporin alkynyl halides, such as cyclosporin alkynyl iodide, can be obtained with *N*-iodosuccinimide instead of *N*-bromosuccinimide. Palladium-catalyzed coupling of cyclosporin alkynyl bromide (or cyclosporin alkynyl iodide) with various alkynes affords cyclosporin diynes of Formula VI smoothly.

Scheme 4



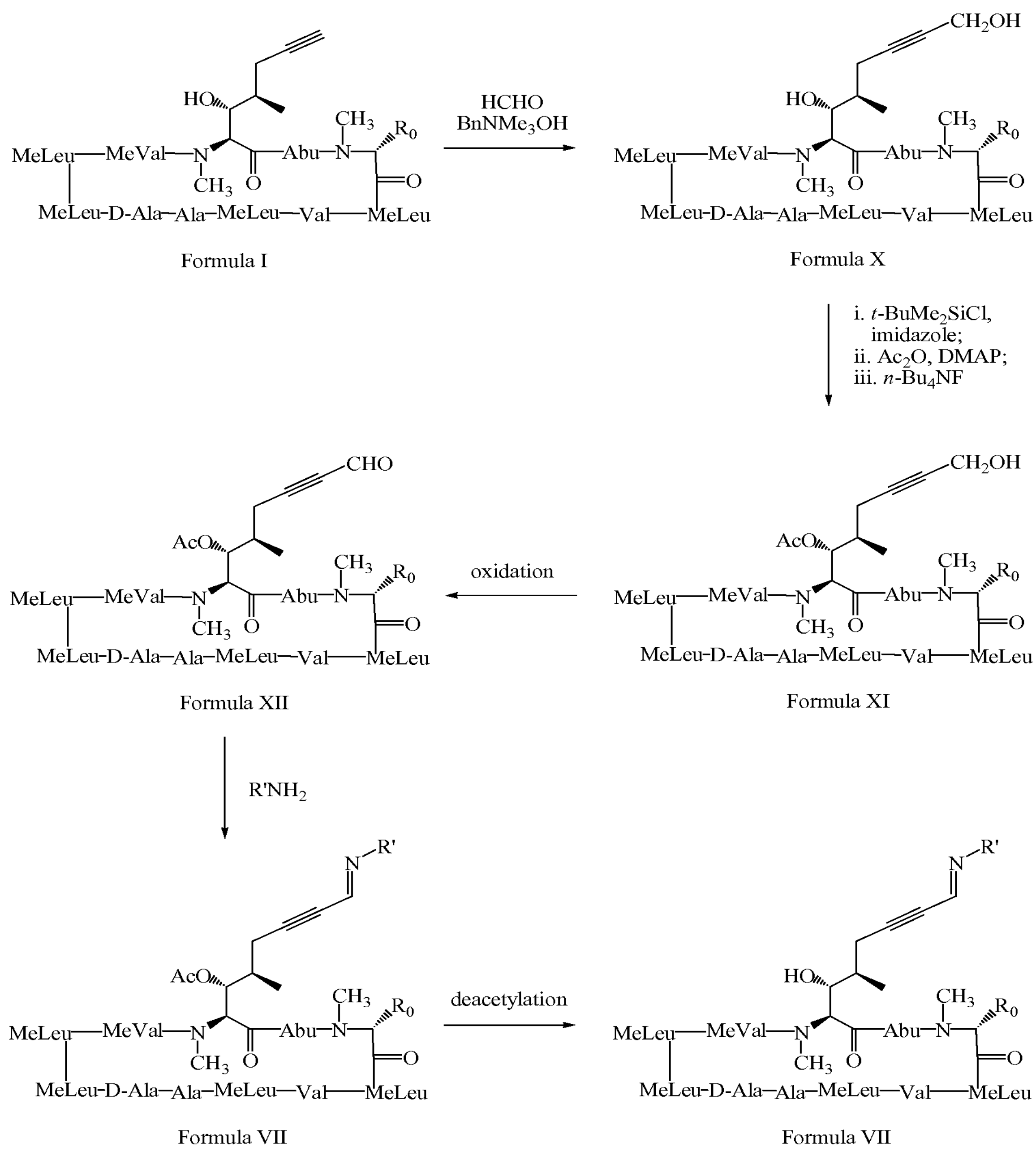
[0044] Another embodiment of the present invention relates to the alkylation of cyclosporine alkyne (Formula I, R_1 = H). As shown in Scheme 5, the treatment of cyclosporine alkyne (Formula I, R_1 = H) with alkyl halides or aldehyde in the presence of a base (cesium carbonate, benzyltrimethylammonium hydroxide, or other strong bases) provides the alkylated cyclosporin alkyne (Formula IX).

Scheme 5



[0045] Another embodiment of the present invention relates to the incorporation of a carbon-nitrogen double bond (C=N) in the cyclosporin alkyne of Formula I. As shown in Scheme 6, the reaction of cyclosporin alkyne (Formula I, R_1 = H) with formaldehyde, using benzyltrimethylammonium hydroxide as a base, provides the cyclosporin diol of Formula X. Selective protection of the primary alcohol of the cyclosporin diol with *tert*-butyldimethylsilyl choloride, followed by acetylation of the second alcohol with acetic anhydride and then desilylation with tetrabutylammonium fluoride, affords the mono-alcohol (Formula XI) smoothly. Swern oxidation of the mono-alcohol affords the cyclosporin aldehyde of Formula XII. Treatment of the aldehyde with hydroxylamine, alkyloxyamines ($RONH_2$), or hydrazines (R_2NNH_2) affords the corresponding cyclosporin oximes (CH=N-OR) and hydrazones (CH=N-NR_2) of Formula VII, respectively.

Scheme 6



[0046] Some of the compounds disclosed in the present invention are useful as immunosuppressive agents. Administration of these compounds suppresses the immune response in organ transplant patients and, thus, prevents allograft rejection. The compounds of the present invention possess enhanced or similar immunosuppressive activity, compared to cyclosporin A. For example, as shown in Figure 1, the cyclosporin alkyne analogue compound disclosed in Example 25

demonstrates immunosuppressive activity two times more potent over cyclosporin A, while the cyclosporin alkyne analogue compound disclosed in Example 10 shows similar potency to cyclosporin A in the concanavalin A (ConA) stimulated murine splenocyte assay. Table 1 shows the immunosuppressive activities of several novel cyclosporin alkyne analogue compounds disclosed in the present application. (The third column in Table 1 contains cyclosporin A positive control values included for comparison.)

Table 1. Immunosuppressive Activities of Novel Cyclosporin Alkyne Analogue Compounds of the Present Invention

Example Where the Novel Cyclosporin Alkyne Analogue Compound is Disclosed	IC_{50} (ng/mL)	IC_{50} (ng/mL) of CsA
Example 7	25	15
Example 10	25	20
Example 13	42	15
Example 14	32	15
Example 22	15	18
Example 25	12	20
Example 27	12	31
Example 29	20	31
Example 33	43	15
Example 35	77	18
Example 38	24	18
Example 43	46	18

[0047] The compounds disclosed in the present invention are useful for the prevention or treatment of viral-induced disorders that are dependent upon the presence of cyclophilin A. The compounds of the present invention used to treat these viral infections may possess potent immunosuppressive activity (via inhibition of calcineurin) or may be completely devoid of immunosuppressive activity (do not inhibit calcineurin). However, the mechanism that the immunosuppressive and non-immunosuppressive cyclosporin compounds share is their activity at cyclophilin A.

[0048] Cyclophilin A enzyme activity, i.e., peptidyl-prolyl cis-trans isomerase activity, is important to the folding and trafficking of proteins. The HIV infectivity of CD4+ T-cells and viral replication are dependent upon the incorporation of cyclophilin A into HIV-1 virions through interactions with the Gag polyprotein.

Inhibition of the cyclophilin A enzyme activity is necessary and sufficient for anti-HIV-1 activity.

[0049] In one embodiment of the present invention, the viral-induced disorder is a human immunodeficiency virus (HIV)-induced disorder. Thus, compounds of the present invention that lack immunosuppressant activity as determined by the Concanavalin A (Con A)-stimulated murine splenocyte assay but retain potent peptidyl prolyl isomerase (PPIase) inhibitory (cyclophilin A) activity may possess anti-HIV activity. In addition, compounds of the present invention that have immunosuppressive activity as determined by the Con A-stimulated murine splenocyte assay and also possess potent PPIase inhibitory (cyclophilin A) activity may possess anti-HIV activity.

[0050] *In vitro* biological assays that allow the determination of binding affinity to cyclophilin A or allow the determination of inhibition of peptidyl cis-trans isomerase activity are described in Handschumacher et al., "Cyclophilin: A Specific Cytosolic Binding Protein for Cyclosporin A," *Science* 226:544-547 (1984) and Kofron et al., "Determination of Kinetic Constants for Peptidyl Prolyl Cis-Trans Isomerases by an Improved Spectrophotometric Assay," *Biochemistry* 30:6127-6134 (1991), respectively, which are both hereby incorporated by reference in their entirety.

[0051] The *in vitro* anti-HIV activity of compounds of the present invention can be measured in established cell line cultures as described by Mayaux et al., "Triterpene Derivatives That Block Entry of Human Immunodeficiency Virus Type 1 Into Cells," *Proc. Natl. Acad. Sci. USA* 91:3564-3568 (1994), which is hereby incorporated by reference in its entirety.

[0052] In another embodiment of the present invention, the compound of the present invention is administered in combination with antiretroviral agents, such as nucleoside reverse transcriptase inhibitors, nonnucleoside reverse transcriptase inhibitors, human immunodeficiency virus protease inhibitors, fusion inhibitors, and combinations thereof. Examples of nucleoside reverse transcriptase inhibitors include, but are not limited to, Zidovudine, Didanosine, Stavudine, and Lamivudine. Examples of nonnucleoside reverse transcriptase inhibitors include, but are not limited to, Nevirapine, Efavirenz, and Delavirdine. Examples of human immunodeficiency virus protease inhibitors include, but are not limited to,

Saquinovir, Indinavir, and Ritonavir. Examples of fusion inhibitors include, but are not limited to, Enfuvirtide.

[0053] Although cyclophilin PPIase activity would appear to be implicated in anti-HCV activity as it is for anti-HIV-1 activity, the hepatitis C virus (HCV) proteins that may interact with cyclophilin A have yet to be identified. In another embodiment of the present invention, the viral-induced disorder is a HCV-induced disorder. Hepatitis C infections or HCV induced disorders are, for example, chronic hepatitis, liver cirrhosis, or liver cancer (e.g., hepatocellular carcinoma). Thus, compounds of the present invention that lack immunosuppressant activity as determined by the Concanavalin A (Con A)-stimulated murine splenocyte assay but retain potent peptidyl prolyl isomerase (PPIase) inhibitory (cyclophilin A) activity may possess anti-HCV activity. In addition, compounds of the present invention that have immunosuppressive activity as determined by the Con A-stimulated murine splenocyte assay and also possess potent PPIase inhibitory (cyclophilin A) activity may possess anti-HCV activity. The compounds of the present invention may also be used as a prophylactic treatment for neonates born to HCV-infected mothers, for healthcare workers exposed to the virus, or for transplant recipients, e.g., organ or tissue transplant (e.g. liver transplant) recipients, to eliminate possible recurrent infection after transplantation.

[0054] In another embodiment of the present invention, the compound of the present invention is administered in combination with an interferon. Examples of interferons include, but are not limited to, interferon α2a and interferon α2b. The interferon can be a pegylated interferon. Examples of interferons include, but are not limited to, pegylated interferon α2a or pegylated interferon α2b.

[0055] Utility of the immunosuppressive or non-immunosuppressive cyclosporin compounds of the present invention in treating diseases or conditions from HCV infection can be demonstrated in standard animal or clinical tests in accordance with the methods described in Examples 54 and 55, for example.

[0056] Some of the compounds disclosed in the present invention also possess utility in the treatment of autoimmune and chronic inflammatory diseases such as asthma, rheumatoid arthritis, multiple sclerosis, psoriasis, and ulcerative colitis, to name only a few.

[0057] The compounds disclosed in the present invention are also useful for the treatment of ocular allergy and dry eye. Allergan is currently marketing a topical formulation of cyclosporin A called Restasis™ (cyclosporin ophthalmic emulsion) for the treatment of keratoconjunctivitis sicca or chronic dry eye syndrome in patients whose tear production is presumed to be suppressed due to ocular inflammation. While the exact mechanism of Restasis™ is unknown, it is thought to act as an immunomodulator with anti-inflammatory effects ("Annual Update 2003: Ophthalmic Drugs" *Drugs of the Future*, 28(3): 287-307 (2003); Clark et al., "Ophthalmic Drug Discovery," *Nature Reviews in Drug Discovery*, 2:448-459 (2003), which are hereby incorporated by reference in their entirety).

[0058] For treatment of the above mentioned diseases, therapeutically effective doses of the compounds of the present invention may be administered orally, topically, parenterally, by inhalation spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants, and vehicles. The term parenteral, as used herein, includes subcutaneous injections, intravenous, intramuscular, intrasternal injection, or infusion techniques.

[0059] The pharmaceutical compositions containing the active ingredient may be in the form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. The pharmaceutical compositions of the present invention contain the active ingredient formulated with one or more pharmaceutically acceptable carriers. As used herein, the term "pharmaceutical acceptable carrier" means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material, or formulation auxillary of any type. Some examples of pharmaceutically acceptable carriers are sugars such as lactose, glucose, and sucrose; starches such as corn starch or potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose, and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil, and soybean oil; glycols such as propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; non-toxic, compatible lubricants such as sodium lauryl

sulfate and magnesium stearate; as well as coloring agents, releasing agents, sweetening, and flavoring and perfuming agents. Preservatives and antioxidants, such as ethyl or n-propyl p-hydroxybenzoate, can also be included in the pharmaceutical compositions.

[0060] Dosage forms for topical or transdermal administration of compounds disclosed in the present invention include ointments, pastes, creams, lotions, gels, plasters, cataplasms, powders, solutions, sprays, inhalants, or patches. The active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers, as may be required. The ointments, pastes, creams and gels may contain, in addition to an active compound of the present invention, excipients such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

[0061] For nasal administration, the compounds disclosed in the present invention can be administered, as suitable, in liquid or powdered form from a nasal applicator. Forms suitable for ophthalmic use will include lotions, tinctures, gels, ointment and ophthalmic inserts, as known in the art. For rectal administration (topical therapy of the colon), the compounds of the present invention may be administered in suppository or enema form, in solution in particular, for Example in vegetable oil or in an oily system for use as a retention enema.

[0062] The compounds disclosed in the present invention may be delivered to the lungs by the inhaled route either in nebulizer form or as a dry powder. The advantage of the inhaled route, over the systemic route, in the treatment of asthma and other diseases of airflow obstruction and/or chronic sinusitis, is that patients are exposed to very small quantities of the drug and the compound is delivered directly to the site of action.

[0063] Dosages of the compounds of the present invention employed for the treatment of the maladies identified in the present invention will vary depending on the site of treatment, the particular condition to be treated, the severity of the condition, the subject to be treated (who may vary in body weight, age, general health, sex, and other factors) as well as the effect desired.

[0064] Dosage levels ranging from about 0.05 mg to about 50 mg per kilogram of body weight per day are useful for the treatment of the conditions or

diseases identified in the present invention. This means the amount of the compound disclosed in the present invention that is administered will range from 2.5 mg to about 2.5 gm per patient per day.

[0065] The amount of active ingredient that may be combined with the pharmaceutical carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a formulation intended for the oral administration of humans may contain from 2.5 mg to 2.5 gm of active compound of the present invention compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to 95 percent of the total composition. Dosage unit forms will generally contain between from about 5 mg to about 500 mg of active compound of the present invention. Dosage for topical preparation will, in general be less (one tenth to one hundredth) of the dose required for an oral preparation.

EXAMPLES

<u>Example 1</u> – Preparation of Cyclosporin Acetate

[0066] A solution of cyclosporin A (5.0 g, 4.16 mmol), acetic anhydride (7.80 mL, 83.2 mmol), and DMAP (760 mg, 6.2 mmol) in methylene chloride (40 mL) was stirred overnight at room temperature under N_2 atmosphere. Saturated sodium bicarbonate solution (200 mL) was added to the solution and stirred for an additional 2 h. The mixture was extracted with ether, washed with 1 N HCl, neutralized with saturated sodium bicarbonate solution, washed with brine, dried over sodium sulfate, and concentrated *in vacuo* to afford cyclosporin acetate (4.92 g, 95%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, J = 9.6 Hz, 1H), 8.04 (d, J = 6.9 Hz, 1H), 7.51 (d, J = 9.4 Hz, 1H), 7.47 (d, J = 7.8 Hz, 1H), 5.67 (dd, J = 11.0, 4.0 Hz, 1H), 5.60–5.44 (m, 2H), 5.39 (dd, J = 11.7, 3.7 Hz, 1H), 5.32–5.13 (m, 4H), 5.06–4.93 (m, 2H), 4.85 (t, J = 7.2 Hz, 1H), 4.77 (t, J = 9.6 Hz, 1H), 4.65 (d, J = 13.7 Hz, 1H), 4.41 (t, J = 7.0 Hz, 1H), 3.46 (s, 3H), 3.26 (s, 3H), 3.24 (s, 3H), 3.21 (s, 3H), 3.10 (s, 3H), 2.68 (s, 3H), 2.66 (s, 3H), 2.50–2.35 (m, 1H), 2.25–1.80 (m, 6H), 2.08 (s, 3H), 2.01 (s, 3H), 1.75–1.55 (m, 6H), 1.45–0.75 (m, 55H); ESI MS *m/z* 1245 $[C_{64}H_{113}N_{11}O_{13} + H]^+$.

<u>Example 2</u> – Preparation of Acetyl Cyclosporin Aldehyde

[0067] Ozone was bubbled into a solution of cyclosporin acetate from Example 1 (3.0 g, 2.4 mmol) in methylene chloride (70 mL) at –78°C until a blue color was developed. The mixture was degassed with nitrogen for a few min and dimethylsulfide (3 mL) was added at –78°C. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. The reaction mixture was concentrated in vacuo and the residue was dissolved in ethyl acetate (300 mL), washed with water (2 × 70 mL) and brine (70 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford acetyl cyclosporin aldehyde (2.79 g, 94%) as a white solid. The crude product was carried to the next step without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.60 (d, J = 3.5 Hz, 1H), 8.55 (d, J = 9.7 Hz, 1H), 7.96 (d, J = 6.8 Hz, 1H), 7.52 (d, J = 7.7 Hz, 1H), 7.46 (d, J = 9.0 Hz, 1H), 5.67 (dd, J = 11.0, 3.8 Hz, 1H), 5.60–5.45 (m, 2H), 5.32 (dd, J = 12.1, 3.3 Hz, 1H), 5.24–5.10 (m, 2H), 5.08–4.90 (m, 2H), 4.84 (t, J = 7.1 Hz, 1H), 4.73 (t, J = 9.6 Hz, 1H), 4.64 (d, J = 13.8 Hz, 1H), 4.41 (t, J = 7.0 Hz, 1H), 3.46 (s, 3H), 3.29 (s, 6H), 3.21 (s, 3H), 3.08 (s, 3H), 2.67 (s, 3H), 2.65 (s, 3H), 2.50–2.35 (m, 2H), 2.25–1.80 (m, 6H), 1.99 (s, 3H), 1.75–1.55 (m, 3H), 1.50–0.75 (m, 57H); ESI MS *m/z* 1233 $[C_{62}H_{109}N_{11}O_{14} + H]^+$.

<u>Example 3</u> – Preparation of Cyclosporin Alkyne

[0068] To a stirred solution of acetyl cyclosporin aldehyde from Example 2 (1.94 g, 1.57 mmol) in methanol (20 mL) was added a solution of dimethyl (1-diazo-2-oxopropyl)phosphonate (3.01 g, 15.7 mmol) in methanol (10 mL) followed by potassium carbonate (2.17 g, 15.7 mmol). The resulting green suspension was stirred at room temperature overnight. The solution was filtered through diatomaceous earth and the filtrate was concentrated. The residue was dissolved in ethyl acetate (300 mL) and washed with water (2 × 100 mL). The combined aqueous layers were extracted with ethyl acetate (100 mL). The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated to dryness. Purification by semi-preparative HPLC gave cyclosporin alkyne (848 mg, 45%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, J = 9.7 Hz, 1H), 7.62 (d, J = 9.3 Hz, 1H), 7.56 (d, J = 6.9 Hz, 1H), 7.34 (d, J = 7.7 Hz, 1H), 5.73−5.68 (m, 1H), 5.57−5.45 (m, 2H), 5.22−4.45 (m, 12H), 4.03−3.98 (m, 1H), 3.49 (s, 3H), 3.38 (s, 3H), 3.24 (s, 3H), 3.09

(s, 3H), 3.08 (s, 3H), 2.72 (s, 3H), 2.70 (s, 3H), 2.50−0.64 (m, 66H); ESI MS *m/z* 1187 $[C_{61}H_{107}N_{11}O_{12} + H]^+$.

Example 4 – Preparation of Cyclosporin Alkyne

[0069] To a −78°C solution of (trimethylsilyl)diazomethane (4.6 mL, 2.0 M solution in Et_2O, 9.2 mmol) in THF (5 mL) was added *n*-BuLi (3.4 mL, 2.5 M solution in hexanes, 8.4 mmol) dropwise. The resulting yellow suspension was stirred for 30 min, and then a solution of acetyl cyclosporine aldehyde from Example 2 (1.03 g, 0.84 mmol) in THF (5 mL) was added dropwise. The mixture was stirred at -78°C for 30 min then warmed to 0°C for 15 min. The reaction was quenched with saturated NH_4Cl. The mixture was partitioned between EtOAc and H_2O. The aqueous layer was extracted with EtOAc. The combined organics were washed with brine, dried over Na_2SO_4, and concentrated. Purification by semi-preparative HPLC gave the cyclosporine alkyne (364 mg, 37%) as a white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, J = 9.7 Hz, 1H), 7.62 (d, J = 9.3 Hz, 1H), 7.56 (d, J = 6.9 Hz, 1H), 7.34 (d, J = 7.7 Hz, 1H), 5.73−5.68 (m, 1H), 5.57−5.45 (m, 2H), 5.22−4.45 (m, 12H), 4.03−3.98 (m, 1H), 3.49 (s, 3H), 3.38 (s, 3H), 3.24 (s, 3H), 3.09 (s, 3H), 3.08 (s, 3H), 2.72 (s, 3H), 2.70 (s, 3H), 2.50−0.64 (m, 66H); ESI MS *m/z* 1187 $[C_{61}H_{107}N_{11}O_{12} + H]^+$.

Example 5 – Preparation of the Acetate of Cyclosporin Alkyne

[0070] To a −78°C solution of (trimethylsilyl)diazomethane (4.5 mL, 2.0 M solution in Et_2O, 8.9 mmol) in THF (10 mL) was added *n*-BuLi (3.2 mL, 2.5 M solution in hexanes, 8.1 mmol) dropwise. The resulting yellow suspension was stirred for 30 min, and then a solution of acetyl cyclosporine aldehyde from Example 2 (1.00 g, 0.81 mmol) in THF (5 mL) was added dropwise. The mixture was stirred at -78°C for 5 min. The reaction was quenched with a mixture of acetic anhydride (1.5 mL, 4.1 mmol) and pyridine (1.4 mL, 4.9 mmol) in THF (5 mL) and then warmed to room temperature for 15 min. The reaction was quenched with saturated NH_4Cl. The mixture was partitioned between Et_2O and H_2O. The aqueous layer was extracted with Et_2O. The combined organics were washed with brine, dried over Na_2SO_4, and concentrated. Purification by semi-preparative HPLC gave the acetate

of cyclosporine alkyne (389 mg, 40%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.46 (d, J = 9.5 Hz, 1H), 8.07 (d, J = 6.9 Hz, 1H), 7.72 (d, J = 9.1 Hz, 1H), 7.54 (d, J = 7.7 Hz, 1H), 5.69 (dd, J = 10.8, 3.6 Hz, 1H), 5.55–5.40 (m, 3H), 5.30 (dd, J = 11.7, 3.6 Hz, 1H), 5.15 (t, J = 6.1 Hz, 1H), 5.02–4.60 (m, 5H), 4.47 (t, J = 6.9 Hz, 1H), 3.46 (s, 3H), 3.28 (s, 3H), 3.23 (s, 3H), 3.20 (s, 3H), 3.07 (s, 3H), 2.69 (s, 3H), 2.67 (s, 3H), 2.45–2.35 (m, 1H), 2.30–2.02 (m, 5H), 2.00 (s, 3H), 1.95–1.55 (m, 8H), 1.45–0.75 (m, 55H); ESI MS m/z 1229 $[C_{63}H_{109}N_{11}O_{13} + H]^+$.

Example 6 – Preparation of the Acetate of Cyclosporin Alkyne

[0071] To a solution of cyclosporine alkyne from Example 3 (0.44 g, 0.37 mmol) in methylene chloride (5 mL) was added pyridine (0.90 mL, 11.1 mmol) followed by DMAP (68 mg, 0.55 mmol) and acetic anhydride (0.70 mL, 7.4 mmol), then the mixture was stirred at room temperature for 1.5 d. The reaction mixture was diluted with ethyl ether (100 mL), washed with a saturated solution of sodium bicarbonate (30 mL) and brine (30 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the acetate of cyclosporine alkyne (0.23 g, 51%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.46 (d, J = 9.5 Hz, 1H), 8.07 (d, J = 6.9 Hz, 1H), 7.72 (d, J = 9.1 Hz, 1H), 7.54 (d, J = 7.7 Hz, 1H), 5.69 (dd, J = 10.8, 3.6 Hz, 1H), 5.55–5.40 (m, 3H), 5.30 (dd, J = 11.7, 3.6 Hz, 1H), 5.15 (t, J = 6.1 Hz, 1H), 5.02–4.60 (m, 5H), 4.47 (t, J = 6.9 Hz, 1H), 3.46 (s, 3H), 3.28 (s, 3H), 3.23 (s, 3H), 3.20 (s, 3H), 3.07 (s, 3H), 2.69 (s, 3H), 2.67 (s, 3H), 2.45–2.35 (m, 1H), 2.30–2.02 (m, 5H), 2.00 (s, 3H), 1.95–1.55 (m, 8H), 1.45–0.75 (m, 55H); ESI MS m/z 1229 $[C_{63}H_{109}N_{11}O_{13} + H]^+$.

Example 7 – Preparation of Cyclosporin yne-ene

[0072] To a mixture of cyclosporin alkyne from Example 3 (55 mg, 0.046 mmol), copper(I) iodide (4 mg, 0.023 mmol), dichlorobis(triphenylphosphine)palladium(II) (16 mg, 0.023 mmol) in triethylamine (2 mL) was added vinyliodide (34 µL, 0.46 mmol), then the mixture was stirred at room temperature for 1 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The crude material was purified by semi-preparative

HPLC to afford cyclosporin yne-ene (24 mg, 43%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (d, J = 9.3 Hz, 1H), 7.75 (d, J = 7.2 Hz, 1H), 7.56 (d, J = 8.4 Hz, 1H), 7.30 (d, J = 7.8 Hz, 1H), 5.80–5.65 (m, 3H), 5.56 (d, J = 2.4 Hz, 1H), 5.52–5.36 (m, 4H), 5.30 (dd, J = 11.4, 3.6 Hz, 1H), 5.20–4.95 (m, 6H), 4.84 (t, J = 7.2 Hz, 2H), 4.72–4.60 (m, 2H), 4.53 (t, J = 7.2 Hz, 1H), 3.88 (t, J = 6.3 Hz, 1H), 3.50 (s, 3H), 3.38 (s, 3H), 3.27 (s, 3H), 3.13 (s, 3H), 3.10 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.45–2.35 (m, 2H), 2.20–1.80 (m, 8H), 1.75–1.55 (m, 5H), 1.45–0.75 (m, 48H); ESI MS m/z 1213 $[C_{63}H_{109}N_{11}O_{12} + H]^+$; HPLC 98.6% (AUC), t_R = 19.32 min.

<u>Example 8</u> – Preparation of *trans*-Cyclosporin yne-ene

[0073] To a mixture of cyclosporin alkyne from Example 3 (65 mg, 0.055 mmol), copper(I) iodide (5 mg, 0.028 mmol), dichlorobis(triphenylphosphine)palladium(II) (20 mg, 0.028 mmol) in triethylamine (2 mL) was added *trans*-1,2-dichloroethylene (85 μL, 1.1 mmol), then the mixture was stirred at room temperature for 4 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford trans-cyclosporin yne-ene (13 mg, 19%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, J = 9.6 Hz, 1H), 7.71 (d, J = 7.2 Hz, 1H), 7.57 (d, J = 8.1 Hz, 1H), 7.27 (d, J = 7.8 Hz, 1H), 6.43 (d, J = 13.6 Hz, 1H), 5.90 (d, J = 13.6 Hz, 1H), 5.70 (dd, J = 10.8, 3.6 Hz, 1H), 5.45 (d, J = 6.3 Hz, 1H), 5.30 (dd, J = 11.7, 3.6 Hz, 1H), 5.17–4.92 (m, 5H), 4.83 (t, J = 6.9 Hz, 1H), 4.75–4.62 (m, 2H), 4.54 (t, J = 7.2 Hz, 1H), 3.85 (t, J = 6.3 Hz, 1H), 3.49 (s, 3H), 3.39 (s, 3H), 3.27 (s, 3H), 3.12 (s, 3H), 3.11 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.45–2.35 (m, 2H), 2.20–1.80 (m, 8H), 1.75–1.55 (m, 5H), 1.45–0.75 (m, 53H); ESI MS m/z 1247 $[C_{63}H_{108}ClN_{11}O_{12} + H]^+$; HPLC >99% (AUC), t_R = 19.92 min.

<u>Example 9</u> – Preparation of the Acetate of *cis*-Cyclosporin yne-ene

[0074] To a mixture of the acetate of cyclosporine alkyne from Example 6 (166 mg, 0.14 mmol), copper(I) iodide (13 mg, 0.068 mmol), dichlorobis(triphenylphosphine)palladium(II) (48 mg, 0.068 mmol) in triethylamine (4 mL) was added *cis*-1,2-dichloroethylene (0.20 mL, 2.7 mmol), then the mixture was stirred at room temperature for 12 h. *Cis*-1,2-dichloroethylene (0.10 mL,

1.3 mmol) was refilled, and the mixture was stirred for 5 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the acetate of *cis*-cyclosporin yne-ene (22 mg, 13%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45 (d, J = 9.6 Hz, 1H), 8.08 (d, J = 7.2 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.59 (d, J = 7.8 Hz, 1H), 6.28 (d, J = 7.4 Hz, 1H), 5.82 (d, J = 7.4 Hz, 1H), 5.70 (dd, J = 10.8, 3.6 Hz, 1H), 5.62–5.10 (m, 5H), 5.03–4.72 (m, 4H), 4.64 (d, J = 13.8 Hz, 1H), 4.48 (t, J = 7.0 Hz, 1H), 3.44 (s, 3H), 3.29 (s, 3H), 3.24 (s, 3H), 3.19 (s, 3H), 3.08 (s, 3H), 2.69 (s, 3H), 2.68 (s, 3H), 2.45–2.35 (m, 1H), 2.30–2.05 (m, 7H), 1.99 (s, 3H), 1.95–1.60 (m, 5H), 1.45–0.75 (m, 55H); ESI MS *m/z* 1289 $[C_{65}H_{110}ClN_{11}O_{13} + H]^+$.

Example 10 – **Preparation of *cis*-Cyclosporin yne-ene**

[0075] To a solution of acetate of *cis*-cyclosporin yne-ene from Example 9 (22 mg, 0.017 mmol) in MeOH (3 mL) was added potassium carbonate (47 mg, 0.34 mmol), then the mixture was stirred at room temperature for 8 h. The reaction mixture was diluted with ethyl acetate (30 mL), then washed with water (10 mL). The aqueous layer was separated and extracted with ethyl acetate (30 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford cis-cyclosporin yne-ene (16 mg, 76%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (d, J = 9.6 Hz, 1H), 7.76 (d, J = 7.2 Hz, 1H), 7.60 (d, J = 8.3 Hz, 1H), 7.33 (d, J = 7.8 Hz, 1H), 6.30 (d, J = 7.3 Hz, 1H), 5.83 (d, J = 7.3 Hz, 1H), 5.71 (dd, J = 11.1, 4.0 Hz, 1H), 5.44 (d, J = 6.7 Hz, 1H), 5.31 (dd, J = 11.5, 3.6 Hz, 1H), 5.17–4.95 (m, 5H), 4.84 (t, J = 7.2 Hz, 1H), 4.75–4.62 (m, 2H), 4.54 (t, J = 7.2 Hz, 1H), 3.92 (t, J = 6.5 Hz, 1H), 3.49 (s, 3H), 3.39 (s, 3H), 3.27 (s, 3H), 3.12 (s, 3H), 3.10 (s, 3H), 2.72 (s, 3H), 2.71 (s, 3H), 2.45–2.25 (m, 2H), 2.20–1.90 (m, 6H), 1.80–1.55 (m, 5H), 1.45–0.75 (m, 55H); ESI MS *m/z* 1247 $[C_{63}H_{108}ClN_{11}O_{12} + H]^+$; HPLC >99% (AUC), t_R = 19.21 min.

Example 11 – **Preparation of the Acetate of *trans*-Cyclosporin yne-ene**

[0076] To a mixture of the acetate of cyclosporin alkyne from Example 6 (74 mg, 0.06 mmol), copper(I) iodide (6 mg, 0.03 mmol),

dichlorobis(triphenylphosphine)palladium(II) (21 mg, 0.03 mmol) in triethylamine (2 mL) was added (2-bromovinyl)trimethylsilane (0.18 mL, 1.2 mmol), then the mixture was stirred at room temperature for 12 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the acetate of *trans*-cyclosporin yne-ene (16 mg, 20%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (d, J = 9.6 Hz, 1H), 8.07 (d, J = 7.2 Hz, 1H), 7.66 (d, J = 8.1 Hz, 1H), 7.54 (d, J = 7.8 Hz, 1H), 6.24 (d, J = 19.2 Hz, 1H), 5.88 (d, J = 19.2 Hz, 1H), 5.70 (dd, J = 10.8, 3.6 Hz, 1H), 5.55–5.10 (m, 6H), 5.03–4.92 (m, 2H), 4.86 (t, J = 7.2 Hz, 1H), 4.76 (t, J = 9.5 Hz, 1H), 4.64 (d, J = 13.9 Hz, 1H), 4.46 (t, J = 7.2 Hz, 1H), 3.44 (s, 3H), 3.31 (s, 3H), 3.25 (s, 3H), 3.20 (s, 3H), 3.07 (s, 3H), 2.69 (s, 3H), 2.67 (s, 3H), 2.45–2.35 (m, 1H), 2.30–2.05 (m, 7H), 1.98 (s, 3H), 1.75–1.55 (m, 3H), 1.45–0.75 (m, 56H), 0.07 (s, 9H); ESI MS *m/z* 1327 $[C_{68}H_{119}N_{11}O_{13}Si + H]^+$.

<u>Example 12</u> – Preparation of *trans*-Cyclosporin yne-ene

[0077] To a solution of the acetate of *trans*-cyclosporin yne-ene from Example 11 (16 mg, 0.012 mmol) in MeOH (2 mL) was added potassium carbonate (41 mg, 0.30 mmol), then the mixture was stirred at room temperature for 8 h. The reaction mixture was diluted with ethyl acetate (30 mL), then washed with water (10 mL). The aqueous layer was separated and extracted with ethyl acetate (30 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford *trans*-cyclosporin yne-ene (6 mg, 40%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (d, J = 9.8 Hz, 1H), 7.78 (d, J = 7.2 Hz, 1H), 7.58 (d, J = 8.2 Hz, 1H), 7.34 (d, J = 7.9 Hz, 1H), 6.30 (d, J = 19.2 Hz, 1H), 5.89 (d, J = 19.2 Hz, 1H), 5.71 (dd, J = 11.3, 4.1 Hz, 1H), 5.39 (d, J = 7.1 Hz, 1H), 5.30 (dd, J = 11.4, 3.4 Hz, 1H), 5.20–4.95 (m, 5H), 4.83 (t, J = 7.2 Hz, 1H), 4.75–4.65 (m, 2H), 4.53 (t, J = 7.2 Hz, 1H), 3.92 (t, J = 6.4 Hz, 1H), 3.49 (s, 3H), 3.38 (s, 3H), 3.27 (s, 3H), 3.13 (s, 3H), 3.10 (s, 3H), 2.72 (s, 3H), 2.71 (s, 3H), 2.60–2.35 (m, 2H), 2.20–1.55 (m, 12H), 1.45–0.75 (m, 54H), 0.07 (s, 9H); ESI MS *m/z* 1285 $[C_{66}H_{117}N_{11}O_{12}Si + H]^+$; HPLC 98.5% (AUC), t_R = 22.71 min.

Example 13 – Preparation of *trans*-Cyclosporin yne-ene

[0078] To a mixture of cyclosporine alkyne from Example 3 (40 mg, 0.03 mmol) in triethylamine (2 mL) and tetrahydrofuran (1 mL) was added dichlorobis(triphenylphosphine)palladium(II) (15 mg, 0.02 mmol), copper(I) iodide (4 mg, 0.02 mmol) and *trans*-1-bromopropene (50 μL, 0.6 mmol), then the mixture was stirred at room temperature for 3 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. Purification twice by semi-preparative HPLC gave *trans*-cyclosporin yne-ene (5.5 mg, 15%) as a yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (d, J = 5.6 Hz, 1H), 7.75–7.67 (m, 3H), 7.49–7.38 (m, 4H), 6.05–5.99 (m, 1H), 5.70 (dd, J = 10.9, 4.2 Hz, 1H), 5.42 (dd, J = 15.8, 1.8 Hz, 1H), 5.36 (d, J = 7.0 Hz, 1H), 5.28 (dd, J = 11.4, 3.5 Hz, 1H), 5.19–5.17 (m, 1H), 5.09 (t, J = 6.7 Hz, 1H), 5.06–4.98 (m, 3H), 4.84 (t, J = 7.2 Hz, 1H), 4.73–4.68 (m, 3H), 4.49 (t, J = 7.3 Hz, 1H), 3.90 (t, J = 6.6 Hz, 1H), 3.49 (s, 3H), 3.38 (s, 3H), 3.27 (s, 3H), 3.13 (s, 3H), 3.09 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.55–1.80 (m, 12H), 1.75–1.56 (m, 13H), 1.50–1.19 (m, 41H); ESI MS *m/z* 1227 $[C_{64}H_{111}N_{11}O_{12} + H]^+$; HPLC >99% (AUC), t_R = 19.76 min.

Example 14 – Preparation of *cis*-Cyclosporin yne-ene

[0079] A mixture of cyclosporin alkyne from Example 3 (80 mg, 0.07 mmol), *cis*-1-bromopropene (300 μL, 3.5 mmol) and copper(I) iodide (14 mg, 0.07 mmol) in triethylamine (3 mL) was stirred until a clear solution formed. Dichlorobis(triphenylphosphine)palladium(II) (51 mg, 0.07 mmol) was added, and then the mixture was stirred at room temperature overnight. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The residue was purified by column chromatography (silica gel, 7:3 hexanes/ethyl acetate to ethyl acetate) to give a brown solid. The solid was further purified twice by semi-preparative HPLC to afford cis-cyclosporin yne-ene (29 mg, 35%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (d, J = 9.5 Hz, 1H), 7.77 (d, J = 7.3 Hz, 1H), 7.51 (d, J = 8.5 Hz, 1H), 7.27 (d, J = 6.4 Hz, 1H), 5.94–5.83 (m, 1H), 5.70 (dd, J = 11.0, 4.2 Hz, 1H), 5.45–5.39 (m, 2H), 5.29 (dd, J = 11.5, 3.8 Hz, 1H), 5.20–5.16 (m, 1H), 5.10–4.97 (m, 4H), 4.88–4.79 (m, 1H), 4.74–4.67 (m, 3H), 4.54–4.47 (m 1H), 3.90 (t, J = 6.5 Hz, 1H), 3.50 (s, 3H), 3.39 (s, 3H), 3.27 (s, 3H), 3.13 (s, 3H), 3.10 (s, 3H),

2.71 (s, 3H), 2.70 (s, 3H), 2.48–0.80 (m, 70H); ESI MS *m/z* 1227 [$C_{64}H_{111}N_{11}O_{12}$ + H]$^+$, HPLC >99% (AUC), t_R = 19.85 min.

<u>Example 15</u> – Preparation of Cyclosporin yne-ene

[0080] To a mixture of cyclosporin alkyne from Example 3 (40 mg, 0.03 mmol) in triethylamine (2 mL) and tetrahydrofuran (1 mL) was added dichlorobis(triphenylphosphine)palladium(II) (15 mg, 0.02 mmol), copper(I) iodide (4 mg, 0.02 mmol) and 2-bromopropene (50 μL, 0.6 mmol), then the mixture was stirred at room temperature for 5 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. Purification twice by semi-preparative HPLC gave cyclosporin yne-ene (4.5 mg, 12%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (d, *J* = 9.0 Hz, 1H), 7.74 (d, *J* = 7.3 Hz, 1H), 7.49 (d, *J* = 8.3 Hz, 1H), 7.22 (d, *J* = 7.8 Hz, 1H), 5.71 (dd, *J* = 10.9, 4.3 Hz, 1H), 5.41 (d, *J* = 6.8 Hz, 1H), 5.35 (dt, *J* = 9.1, 5.8 Hz, 1H), 5.27 (dd, *J* = 11.5, 3.7 Hz, 1H), 5.20–5.18 (m, 3H), 5.14 (s, 1H), 5.08 (t, *J* = 6.9 Hz, 1H), 5.05–4.98 (m, 2H), 4.84 (app quintet, *J* = 6.9 Hz, 1H), 4.73–4.67 (m, 2H), 4.52 (app quintet, *J* = 7.4 Hz, 1H), 3.88 (t, *J* = 6.5 Hz, 1H), 3.50 (s, 3H), 3.38 (s, 3H), 3.28 (s, 3H), 3.21–3.18 (m, 1H), 3.13 (s, 3H), 3.10 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.60 (dd, *J* = 17.1, 3.9 Hz, 1H), 2.46–2.35 (m, 1H), 2.63 (t, *J* = 7.7 Hz, 1H), 2.18–2.07 (m, 6H), 2.05–1.96 (m, 3H), 1.90–1.82 (m, 5H), 1.81–1.59 (m, 5H), 1.52–1.38 (m, 4H), 1.36–1.23 (m, 13H), 1.01–0.84 (m, 30H); ESI MS *m/z* 1227 [$C_{64}H_{111}N_{11}O_{12}$ + H]$^+$; HPLC 98.8% (AUC), t_R = 19.82 min.

<u>Example 16</u> – Preparation of Cyclosporin yne-ene

[0081] To a mixture of cyclosporin alkyne from Example 3 (80 mg, 0.07 mmol) and copper(I) iodide (13 mg, 0.07 mmol) in triethylamine (3 mL) was added bromostyrene (a mixture of cis and trans isomers, 180 μL, 1.4 mmol) then the mixture was stirred until a clear solution formed.

Dichlorobis(triphenylphosphine)palladium(II) (50 mg, 0.07 mmol) was added, and then the mixture was stirred at room temperature overnight. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The residue was purified by column chromatography (silica gel, 7:3 hexanes/ethyl acetate to ethyl acetate) to give a brown solid. The solid was further purified twice by semi-

preparative HPLC to afford cyclosporin yne-ene (17 mg, 19%) as a white solid and a mixture of isomers (*cis*/*trans* ~ 1:4 by ^{1}H NMR): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 (d, J = 9.7 Hz, 1H), 7.81 (d, J = 7.4 Hz, 1H), 7.71 (d, J = 7.4 Hz, 1H), 7.47 (d, J = 8.5 Hz, 1H), 7.38–7.29 (m, 12H), 6.94–6.83 (m, 4H), 5.70 (dd, J = 11.0, 4.3 Hz, 1H), 5.44 (d, J = 6.9 Hz, 2H), 5.19–5.15 (m, 2H), 5.08 (t, J = 7.3 Hz, 1H), 5.06–5.00 (m, 6H), 4.83 (t, J = 7.7 Hz, 1H), 4.73–4.69 (m, 4H), 4.55–4.40 (m, 3H), 3.92 (t, J = 6.6 Hz, 1H), 3.63 (s, 3H), 3.52 (s, 3H), 3.40 (s, 3H), 3.29 (s, 3H), 3.12 (s, 3H), 3.11 (s, 3H), 2.50–1.87 (m, 10H), 1.84–1.57 (m, 3H), 1.55–1.19 (m, 10H), 1.08–0.70 (m, 31H); ESI MS *m/z* 1289 $[C_{69}H_{113}N_{11}O_{12} + H]^+$; HPLC 98.2% (AUC), t_R = 20.66 min.

<u>Example 17</u> – Preparation of Phenyl Cyclosporin Alkyne

[0082] A mixture of cyclosporin alkyne from Example 3 (80 mg, 0.07 mmol) in triethylamine (3 mL) was degassed with N_2 for 5 min. Dichlorobis(triphenylphosphine)palladium(II) (28 mg, 0.04 mmol), copper(I) iodide (8 mg, 0.04 mmol) and iodobenzene (80 μL, 0.70 mmol) were added, then the mixture was stirred at room temperature for 1.5 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. Purification by semi-preparative HPLC gave cyclosporine phenyl alkyne (7 mg, 8%) as a brown solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.18 (d, J = 9.8 Hz, 1H), 7.78 (d, J = 7.4 Hz, 1H), 7.71–7.68 (m, 1H), 7.51 (d, J = 8.3 Hz, 1H), 7.40–7.37 (m, 4H), 7.20 (d, J = 7.9 Hz, 1H), 5.69 (dd, J = 10.6, 3.7 Hz, 1H), 5.45 (d, J = 6.9 Hz, 1H), 5.32–5.27 (m, 1H), 5.20–5.16 (m, 1H), 5.09–4.97 (m, 4H), 4.83 (t, J = 7.1 Hz, 1H), 4.74–4.67 (m, 2H), 4.52 (t, J = 7.3 Hz, 1H), 3.92 (t, J = 6.5 Hz, 1H), 3.53 (s, 3H), 3.40 (s, 3H), 3.30 (s, 3H), 3.22–3.17 (m, 2H), 3.13 (s, 3H), 3.10 (s, 3H), 2.70 (s, 3H), 2.69 (s, 3H), 2.53–2.35 (m, 2H), 2.29–1.91 (m, 10H), 1.83–1.83 (m, 11H), 1.50–1.18 (m, 11H), 1.10–0.76 (m, 32H); ESI MS *m/z* 1263 $[C_{67}H_{111}N_{11}O_{12} + H]^+$; HPLC >99% (AUC), t_R = 20.01 min.

<u>Example 18</u> – Preparation of 4-Methoxyphenyl Cyclosporin Alkyne

[0083] A mixture of cyclosporin alkyne from Example 3 (80 mg, 0.07 mmol), 1-iodo-4-methoxybenzene (150 μL, 1.4 mmol) and copper(I) iodide (13 mg, 0.07 mmol) in triethylamine (3 mL) was stirred until a clear solution formed. Dichlorobis(triphenylphosphine)palladium(II) (50 mg, 0.07 mmol) was added, and

then the mixture was stirred at room temperature overnight. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The residue was purified by column chromatography (silica gel, 7:3 hexanes/ethyl acetate to ethyl acetate) to give a brown solid. The solid was further purified twice by semi-preparative HPLC to afford 4-methoxyphenyl cyclosporin alkyne (23 mg, 26%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17 (d, J = 9.7 Hz, 1H), 7.77 (d, J = 7.4 Hz, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.31 (d, J = 8.8 Hz, 2H), 7.21 (d, J = 7.9 Hz, 1H), 6.80 (d, J = 8.8 Hz, 2H), 5.68 (dd, J = 11.0, 4.3 Hz, 1H), 5.42 (d, J = 7.1 Hz, 1H), 5.29 (dd, J = 11.6, 3.9 Hz, 1H), 5.20–5.18 (m, 2H), 5.09–5.05 (m, 2H), 5.01 (dd, J = 16.2, 8.1 Hz, 1H), 4.85 (app quintet, J = 6.9 Hz, 1H), 4.73–4.69 (m, 2H), 4.51 (app quintet, J = 7.2 Hz, 1H), 3.92 (d, J = 6.4 Hz, 1H), 3.80 (s, 3H), 3.52 (s, 3H), 3.40 (s, 3H), 3.29 (s, 3H), 3.20–3.14 (m, 6H), 3.13 (s, 3H), 3.10 (s, 3H), 2.69 (s, 3H), 2.67 (s, 3H), 2.44–1.95 (m, 12H), 1.76–1.61 (m, 3H), 1.45–1.24 (m, 16H), 1.07 (t, J = 8.0 Hz, 4H), 0.97–0.79 (m, 27H); ESI MS m/z 1293 $[C_{68}H_{113}N_{11}O_{13} + H]^+$; HPLC >99% (AUC), t_R = 19.59 min.

<u>Example 19</u> – Preparation of 4-Fluorophenyl Cyclosporin Alkyne

[0084] A mixture of cyclosporin alkyne from Example 3 (80 mg, 0.07 mmol), 4-fluoro-1-iodobenzene (160 μL, 1.4 mmol) and copper(I) iodide (14 mg, 0.07 mmol) in triethylamine (3 mL) was stirred until a clear solution formed. Dichlorobis(triphenylphosphine)palladium(II) (50 mg, 0.07 mmol) was added, and then the mixture was stirred at room temperature overnight. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The residue was purified by column chromatography (silica gel, 7:3 hexanes/ethyl acetate to ethyl acetate) to give a brown solid. The solid was further purified twice by semi-preparative HPLC to afford 4-fluorophenyl cyclosporin alkyne (8.9 mg, 10%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (d, J = 9.8 Hz, 1H), 7.76 (d, J = 7.4 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.35 (ddd, J = 8.7, 5.4, 2.0 Hz, 2H), 7.19 (d, J = 7.9 Hz, 1H), 6.97 (dd, J = 8.7, 8.7 Hz, 2H), 5.68 (dd, J = 11.1, 4.1 Hz, 1H), 5.45 (d, J = 6.7 Hz, 1H), 5.27 (dd, J = 11.5, 3.7 Hz, 1H), 5.18–5.14 (m, 1H), 5.09–4.94 (m, 4H), 4.88–4.79 (m, 1H), 4.74–4.66 (m, 2H), 4.56–4.47 (m, 1H), 3.91 (t, J = 6.4 Hz, 1H), 3.52 (s, 3H), 3.40 (s, 3H), 3.29 (s, 3H), 3.13 (s, 3H), 3.10 (s, 3H), 2.71 (s, 3H), 2.69

(s, 3H), 2.45–0.76 (m, 68H); ESI MS *m/z* 1280 $[C_{67}H_{110}FN_{11}O_{12} + H]^+$; HPLC >99% (AUC), t_R = 20.18 min.

Example 20 – Preparation of Thiophen-2-yl Cyclosporin Alkyne

[0085] A mixture of cyclosporin alkyne from Example 3 (80 mg, 0.07 mmol), 2-iodothiophene (328 mg, 1.4 mmol) and copper(I) iodide (13 mg, 0.07 mmol) in triethylamine (3 mL) was stirred until a clear solution formed. Dichlorobis(triphenylphosphine)palladium(II) (50 mg, 0.07 mmol) was added, and then the mixture was stirred at room temperature overnight. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The residue was purified by column chromatography (silica gel, 7:3 hexanes/ethyl acetate to ethyl acetate) to give a light brown solid. The solid was further purified twice by semi-preparative HPLC to afford thiophen-2-yl cyclosporin alkyne (10.7 mg, 12%) as a white solid: ^1H NMR (500 MHz, $CDCl_3$) δ 8.07 (d, *J* = 9.6 Hz, 1H), 7.74 (d, *J* = 7.4 Hz, 1H), 7.54 (d, *J* = 8.3 Hz, 1H), 7.25 (d, *J* = 5.6 Hz, 1H), 7.15 (dd, *J* = 5.2, 1.0 Hz, 1H), 7.11 (dd, *J* = 3.5, 0.9 Hz, 1H), 6.92 (dd, *J* = 5.1, 3.6 Hz, 1H), 5.69 (dd, *J* = 11.0, 4.3 Hz, 1H), 5.47 (d, *J* = 6.4 Hz, 1H), 5.29 (dd, *J* = 11.4, 3.6 Hz, 1H), 5.18–5.16 (m, 1H), 5.07 (t, *J* = 6.9 Hz, 1H), 5.05–5.00 (m, 2H), 4.86–4.81 (m, 1H), 4.74–4.67 (m, 2H), 4.57–4.51 (m, 1H), 3.90 (t, *J* = 6.4 Hz, 1H), 3.52 (s, 3H), 3.39 (s, 3H), 3.28 (s, 3H), 3.25–3.15 (m, 3H), 3.12 (s, 3H), 3.10 (s, 3H), 3.07–2.90 (m, 4H), 2.82–2.75 (m, 1H), 2.72 (s, 3H), 2.70 (s, 3H), 2.44–1.90 (m, 8H), 1.79–1.58 (m, 6H), 1.48–1.18 (m, 11H), 1.05–0.80 (m, 36H); ESI MS *m/z* 1269 $[C_{65}H_{109}N_{11}O_{12}S + H]^+$; HPLC 98.8% (AUC), t_R = 19.76 min.

Example 21 – Preparation of the Acetate of Cyclosporin Diyne

[0086] To a solution of the acetate of cyclosporin alkyne from Example 6 (90 mg, 0.073 mmol) in pyrrolidine (1 mL) were added copper(I) iodide (7 mg, 0.037 mmol) and dichlorobis(triphenylphosphine)palladium(II) (26 mg, 0.037 mmol), then the mixture was stirred for 5 min at room temperature. 1-Butynyl iodide (145 μL, 1.46 mmol) was added dropwise, and then the mixture was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate (40 mL) and washed with a saturated solution of ammonium chloride (20 mL). The aqueous

layer was extracted with ethyl acetate (2 × 20 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the desired acetate of cyclosporin diyne (15 mg, 16%) as a brown solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (d, J = 9.6 Hz, 1H), 8.05 (d, J = 6.9 Hz, 1H), 7.60 (d, J = 9.0 Hz, 1H), 7.55 (d, J = 7.8 Hz, 1H), 5.70 (dd, J = 10.8, 3.9 Hz, 1H), 5.58–5.35 (m, 2H), 5.30 (dd, J = 12.0, 3.3 Hz, 2H), 5.15 (t, J = 6.9 Hz, 1H), 5.05–4.80 (m, 3H), 4.73 (t, J = 9.6 Hz, 1H), 4.64 (d, J = 13.8 Hz, 1H), 4.44 (t, J = 6.9 Hz, 1H), 3.43 (s, 3H), 3.32 (s, 3H), 3.27 (s, 3H), 3.20 (s, 3H), 3.07 (s, 3H), 2.68 (s, 3H), 2.66 (s, 3H), 2.50–2.35 (m, 1H), 2.30–1.80 (m, 10H), 2.04 (s, 3H), 1.75–1.55 (m, 3H), 1.45–0.75 (m, 59H); ESI MS m/z 1281 $[C_{67}H_{113}N_{11}O_{13} + H]^{+}$.

<u>Example 22</u> – Preparation of Cyclosporin Diyne

[0087] To a solution of the acetate of cyclosporin diyne from Example 21 (18 mg, 0.014 mmol) in MeOH (2 mL) was added potassium carbonate (39 mg, 0.28 mmol), then the mixture was stirred overnight at room temperature. The reaction mixture was quenched with a saturated solution of ammonium chloride, and then extracted with ethyl acetate (3 × 30 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford cyclosporin diyne (9 mg, 53%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (d, J = 9.3 Hz, 1H), 7.73 (d, J = 7.2 Hz, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.23 (d, J = 8.1 Hz, 1H), 5.71 (dd, J = 10.8, 3.9 Hz, 1H), 5.41 (d, J = 6.6 Hz, 1H), 5.28 (dd, J = 11.7, 3.9 Hz, 1H), 5.20–4.95 (m, 5H), 4.83 (t, J = 7.2 Hz, 1H), 4.78–4.63 (m, 2H), 4.52 (t, J = 7.2 Hz, 1H), 3.90 (t, J = 6.3 Hz, 1H), 3.50 (s, 3H), 3.37 (s, 3H), 3.28 (s, 3H), 3.12 (s, 3H), 3.09 (s, 3H), 2.72 (s, 3H), 2.70 (s, 3H), 2.55–2.05 (m, 7H), 1.90–0.80 (m, 66H); ESI MS m/z 1238 $[C_{65}H_{111}N_{11}O_{12} + H]^{+}$; HPLC >99% (AUC), t_R = 20.06 min.

<u>Example 23</u> – Preparation of the Acetate of Cyclosporin Alkynyl Bromide

[0088] To a solution of the acetate of cyclosporin alkyne from Example 6 (0.22 g, 0.18 mmol) in acetone (5 mL) was added *N*-bromosuccinimide (64 mg, 0.36mmol) followed by silver nitrate (6 mg, 0.036 mmol). The reaction flask was

wrapped with aluminum foil. The reaction mixture was stirred at room temperature for 1 h, then poured into ice-water (20 mL) and extracted with ethyl ether (3 × 40 mL). The combined organics were washed with brine (30 mL), dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the acetate of cyclosporin alkynyl bromide (0.23 g, 98%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (d, J = 9.6 Hz, 1H), 8.07 (d, J = 6.9 Hz, 1H), 7.80 (d, J = 8.9 Hz, 1H), 7.63 (d, J = 7.7 Hz, 1H), 5.70 (dd, J = 10.8, 3.6 Hz, 1H), 5.60–5.15 (m, 5H), 5.02–4.80 (m, 4H), 4.76 (d, J = 9.3 Hz, 1H), 4.65 (d, J = 13.9 Hz, 1H), 4.48 (t, J = 7.0 Hz, 1H), 3.43 (s, 3H), 3.30 (s, 3H), 3.25 (s, 3H), 3.19 (s, 3H), 3.10 (s, 3H), 2.69 (s, 3H), 2.68 (s, 3H), 2.45–2.35 (m, 1H), 2.30–2.05 (m, 7H), 2.02 (s, 3H), 1.75–1.55 (m, 4H), 1.45–0.75 (m, 55H); ESI MS m/z 1307 $[C_{63}H_{108}BrN_{11}O_{13} + H]^+$.

<u>Example 24</u> – Preparation of the Acetate of Cyclosporin(trimethylsilyl)diyne

[0089] To a solution of the acetate of cyclosporin alkynyl bromide from Example 23 (20 mg, 0.015 mmol) in pyrrolidine (1 mL) was added (trimethylsilyl)acetylene (42 μL, 0.30 mmol) followed by copper(I) iodide (3 mg, 0.015 mmol) and dichlorobis(triphenylphosphine)palladium(II) (6 mg, 0.008 mmol), then the mixture was stirred at room temperature for 1 h. The reaction was quenched with a saturated solution of ammonium chloride, and then extracted with ethyl acetate (3 × 30 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the acetate of cyclosporin (trimethylsilyl)diyne (6 mg, 30%) as a pale-brown solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (d, J = 9.6 Hz, 1H), 8.05 (d, J = 6.7 Hz, 1H), 7.65 (d, J = 8.8 Hz, 1H), 7.57 (d, J = 7.9 Hz, 1H), 5.70 (dd, J = 11.2, 3.8 Hz, 1H), 5.55–5.35 (m, 2H), 5.29 (td, J = 11.9, 3.7 Hz, 2H), 5.16 (d, J = 6.1 Hz, 1H), 5.03–4.80 (m, 3H), 4.72 (t, J = 9.4 Hz, 1H), 4.63 (d, J = 13.9 Hz, 1H), 4.46 (t, J = 7.0 Hz, 1H), 3.42 (s, 3H), 3.32 (s, 3H), 3.27 (s, 3H), 3.20 (s, 3H), 3.08 (s, 3H), 2.68 (s, 3H), 2.67 (s, 3H), 2.45–2.05 (m, 7H), 2.04 (s, 3H), 1.75–1.55 (m, 3H), 1.45–0.75 (m, 56H), 0.15 (s, 9H); ESI MS m/z 1325 $[C_{68}H_{117}N_{11}O_{13}Si + H]^+$.

<u>Example 25</u> – Preparation of Cyclosporin Diyne

[0090] To a solution of the acetate of cyclosporin (trimethylsilyl)diyne from Example 24 (9 mg, 0.007mmol) in MeOH (2 mL) was added potassium carbonate (19 mg, 0.14 mmol), then the mixture was stirred overnight at room temperature. The reaction mixture was quenched with a saturated solution of ammonium chloride, and then extracted with ethyl acetate (3 × 30 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford cyclosporine diyne (6 mg, 71%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, *J* = 9.7 Hz, 1H), 7.65 (d, *J* = 7.4 Hz, 1H), 7.38 (d, *J* = 8.3 Hz, 1H), 7.17 (d, *J* = 7.9 Hz, 1H), 5.71 (dd, *J* = 10.8, 3.8 Hz, 1H), 5.48 (d, *J* = 6.2 Hz, 1H), 5.27 (dd, *J* = 11.5, 3.9 Hz, 1H), 5.15–4.95 (m, 5H), 4.83 (t, *J* = 7.1 Hz, 1H), 4.75–4.62 (m, 2H), 4.53 (t, *J* = 7.2 Hz, 1H), 3.89 (t, *J* = 6.2 Hz, 1H), 3.51 (s, 3H), 3.38 (s, 3H), 3.28 (s, 3H), 3.11 (s, 3H), 3.10 (s, 3H), 2.70 (s, 3H), 2.69 (s, 3H), 2.60–2.35 (m, 2H), 2.20–0.80 (m, 67H); ESI MS *m/z* 1211 $[C_{63}H_{107}N_{11}O_{12} + H]^+$; HPLC 98.5% (AUC), t_R = 18.78 min.

<u>Example 26</u> – Preparation of the Acetate of Cyclosporin Diyne

[0091] To an ice-cooled solution of 1-propynylmagnesium bromide (0.54 mL, 0.5 M in THF, 0.27 mmol) in THF (1 mL) was added a solution of zinc chloride (0.27 mL, 1 M in ethyl ether, 0.27 mmol). The reaction was stirred at 0°C for 10 min, and then allowed to warm to room temperature. A solution of the acetate of cyclosporin alkynyl bromide from Example 23 (35 mg, 0.027 mmol) in THF (1 mL) was added into the reaction mixture followed by dichlorobis(triphenylphosphine)palladium(II) (10 mg, 0.014 mmol). The resulting reaction mixture was stirred at room temperature for 1.5 h, and then quenched with a saturated solution of ammonium chloride (10 mL). The aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the acetate of cyclosporin diyne (14 mg, 41%) as a pale-brown solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.42 (d, *J* = 9.5 Hz, 1H), 8.07 (d, *J* = 7.0 Hz, 1H), 7.82 (d, *J* = 9.0 Hz, 1H), 7.64 (d, *J* = 7.5 Hz, 1H), 5.71 (dd, *J* = 11.0, 4.5 Hz, 1H), 5.58–5.37 (m, 3H), 5.27 (dd, *J* = 12.0, 4.0 Hz, 1H), 5.17 (t, *J* = 6.0 Hz,

1H), 5.03–4.93 (m, 3H), 4.87 (t, J = 7.0 Hz, 1H), 4.81 (t, J = 9.5 Hz, 1H), 4.65 (d, J = 13.5 Hz, 1H), 4.49 (t, J = 7.0 Hz, 1H), 3.43 (s, 3H), 3.29 (s, 3H), 3.23 (s, 3H), 3.19 (s, 3H), 3.09 (s, 3H), 2.70 (s, 3H), 2.68 (s, 3H), 2.45–2.35 (m, 1H), 2.28–2.04 (m, 7H), 2.03 (s, 3H), 2.02–1.92 (m, 2H), 1.88 (s, 3H), 1.75–1.62 (m, 4H), 1.45–0.75 (m, 53H); ESI MS m/z 1267 $[C_{66}H_{111}N_{11}O_{13} + H]^+$.

Example 27 – Preparation of Cyclosporin Diyne

[0092] To a solution of the acetate of cyclosporin diyne from Example 26 (14 mg, 0.011 mmol) in MeOH (2 mL) was added potassium carbonate (30 mg, 0.22 mmol), then the mixture was stirred overnight at room temperature. The reaction mixture was quenched with a saturated solution of ammonium chloride, and then extracted with ethyl acetate (3 × 30 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford cyclosporin diyne (8 mg, 62%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (d, J = 9.5 Hz, 1H), 7.71 (d, J = 7.0 Hz, 1H), 7.51 (d, J = 8.2 Hz, 1H), 7.37 (d, J = 7.4 Hz, 1H), 5.71 (dd, J = 11.0, 2.9 Hz, 1H), 5.41 (d, J = 6.5 Hz, 1H), 5.38–5.27 (m, 1H), 5.15–4.95 (m, 4H), 4.84 (t, J = 7.0 Hz, 1H), 4.75–4.65 (m, 2H), 4.54 (t, J = 6.9 Hz, 1H), 3.94 (t, J = 6.5 Hz, 1H), 3.48 (s, 3H), 3.37 (s, 3H), 3.25 (s, 3H), 3.12 (s, 3H), 3.10 (s, 3H), 2.72 (s, 3H), 2.71 (s, 3H), 2.55–2.05 (m, 7H), 1.88 (s, 3H), 1.75–0.75 (m, 62H); ESI MS m/z 1225 $[C_{64}H_{109}N_{11}O_{12} + H]^+$; HPLC >99% (AUC), t_R = 19.38 min.

Example 28 – Preparation of the Acetate of Cyclosporin Cyclopropyl Diyne

[0093] To an ice-cooled solution of cyclopropyl(trimethylsilyl)acetylene (37 mg, 0.27 mmol) in triethylamine (1 mL) was added tetrabutylammonium fluoride (0.32 mL, 1 M in THF, 0.32 mmol), then the mixture was stirred for 10 min. The reaction mixture was allowed to warm to room temperature, then a solution of the acetate of cyclosporin alkynyl bromide from Example 23 (35 mg, 0.027 mmol) in triethylamine (1 mL) was added into the mixture followed by copper(I) iodide (3 mg, 0.014 mmol) and dichlorobis(triphenylphosphine)palladium(II) (10 mg, 0.014 mmol). The resulting reaction mixture was stirred at room temperature for 5 h. The reaction mixture was diluted with ethyl acetate (30 mL) and washed with a saturated solution

of ammonium chloride (10 mL). The aqueous layer was extracted with ethyl acetate (2 × 20 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the acetate of cyclosporin cyclopropyl diyne (23 mg, 66%) as a pale-brown solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45 (d, J = 9.4 Hz, 1H), 8.05 (d, J = 6.8 Hz, 1H), 7.71 (d, J = 9.0 Hz, 1H), 7.60 (d, J = 7.8 Hz, 1H), 5.70 (dd, J = 10.9, 3.6 Hz, 1H), 5.58–5.23 (m, 4H), 5.16 (d, J = 5.5 Hz, 1H), 5.02–4.80 (m, 3H), 4.77 (t, J = 9.6 Hz, 1H), 4.64 (d, J = 13.9 Hz, 1H), 4.46 (t, J = 7.0 Hz, 1H), 3.43 (s, 3H), 3.30 (s, 3H), 3.25 (s, 3H), 3.19 (s, 3H), 3.08 (s, 3H), 2.69 (s, 3H), 2.67 (s, 3H), 2.45–2.35 (m, 1H), 2.30–2.03 (m, 6H), 2.02 (s, 3H), 1.75–1.60 (m, 3H), 1.45–0.70 (m, 63H); ESI MS m/z 1293 $[C_{68}H_{113}N_{11}O_{13} + H]^+$.

<u>Example 29</u> – Preparation of Cyclosporin Cyclopropyl Diyne

[0094] To a solution of the acetate of cyclosporin cyclopropyl diyne from Example 28 (20 mg, 0.015 mmol) in MeOH (2 mL) was added potassium carbonate (41 mg, 0.30 mmol), then the mixture was stirred overnight at room temperature. The reaction mixture was quenched with a saturated solution of ammonium chloride, and then extracted with ethyl acetate (3 × 30 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford cyclosporin cyclopropyl diyne (16 mg, 84%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (d, J = 9.7 Hz, 1H), 7.70 (d, J = 7.3 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.25 (d, J = 8.7 Hz, 1H), 5.71 (dd, J = 11.0, 4.0 Hz, 1H), 5.41 (d, J = 6.6 Hz, 1H), 5.28 (dd, J = 11.4, 3.9 Hz, 1H), 5.15–4.95 (m, 4H), 4.83 (t, J = 7.2 Hz, 1H), 4.75–4.65 (m, 3H), 4.52 (t, J = 7.2 Hz, 1H), 3.90 (t, J = 6.4 Hz, 1H), 3.49 (s, 3H), 3.37 (s, 3H), 3.28 (s, 3H), 3.12 (s, 3H), 3.09 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.55–2.30 (m, 2H), 2.20–0.75 (m, 71H); ESI MS m/z 1251 $[C_{66}H_{111}N_{11}O_{12} + H]^+$; HPLC >99% (AUC), t_R = 19.98 min.

<u>Example 30</u> – Preparation of the Acetate of Cyclosporin Diyne

[0095] A mixture of the acetate of cyclosporine alkynyl bromide from Example 23 (40 mg, 0.03 mmol), dichlorobis(triphenylphosphine)palladium(II) (14 mg, 0.02 mmol), copper(I) iodide (4 mg, 0.02 mmol) and phenylacetylene (30 μL,

0.30 mmol) in triethylamine (2 mL) was stirred at room temperature overnight. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. Purification by column chromatography (silica gel, 100% hexanes followed by 100% EtOAc) followed by semi-preparative HPLC gave the acetate of cyclosporin diyne (11 mg, 27%) as a brown solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (d, J = 9.7 Hz, 1H), 8.05 (d, J = 6.9 Hz, 1H), 7.62–7.54 (m, 2H), 7.47–7.44 (m, 2H), 7.34–7.28 (m, 3H), 5.69 (dd, J = 10.9, 3.9 Hz, 1H), 5.59–5.50 (m, 1H), 5.47–5.43 (m, 1H), 5.35 (t, J = 3.5 Hz, 1H), 5.31 (t, J = 3.4 Hz, 1H), 5.15 (t, J = 6.2 Hz, 1H), 5.02–4.91 (m, 4H), 4.86 (t, J = 7.2 Hz, 1H), 4.75 (t, J = 9.6 Hz, 1H), 4.67–4.63 (m, 1H), 4.45 (t, J = 7.0 Hz, 1H), 3.44 (s, 3H), 3.42 (s, 3H), 3.28 (s, 3H), 3.20 (s, 3H), 3.08 (s, 3H), 2.69 (s, 3H), 2.66 (s, 3H), 2.52–2.11 (m, 8H), 2.07 (s, 3H), 1.75–1.55 (m, 4H), 1.41–1.18 (m, 20H), 1.05–0.82 (m, 34H); ESI MS m/z 1329 $[C_{71}H_{113}N_{11}O_{13} + H]^+$.

<u>Example 31</u> – Preparation of Cyclosporin Diyne

[0096] To a solution of the acetate of cyclosporin diyne from Example 30 (11 mg, 0.008 mmol) in MeOH (1 mL) was added potassium carbonate (11 mg, 0.08 mmol) and then the mixture was stirred at room temperature overnight. The mixture was diluted with EtOAc, washed with H_2O (2 ×), brine, dried over Na_2SO_4, and concentrated. Purification by semi-preparative HPLC gave the cyclosporin diyne (6.6 mg, 64%) as a brown solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (d, J = 9.7 Hz, 1H), 7.69 (d, J = 7.5 Hz, 1H), 7.50–7.47 (m, 2H), 7.41 (d, J = 8.3 Hz, 1H), 7.32–7.28 (m, 3H), 7.22 (d, J = 7.9 Hz, 1H), 5.70 (dd, J = 11.0, 4.1 Hz, 1H), 5.48 (d, J = 6.4 Hz, 1H), 5.34–5.29 (m, 2H), 5.15–4.99 (m, 6H), 4.83 (t, J = 7.0 Hz, 1H), 4.74–4.66 (m, 2H), 4.52 (t, J = 7.3 Hz, 1H), 3.93 (t, J = 6.3 Hz, 1H), 3.52 (s, 3H), 3.39 (s, 3H), 3.26 (s, 3H), 3.24–3.17 (m, 3H), 3.11 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.31–2.06 (m, 12H), 1.67–1.62 (m, 4H), 1.55–1.21 (m, 12H), 1.07–0.84 (m, 38H); ESI MS m/z 1287 $[C_{69}H_{111}N_{11}O_{12} + H]^+$; HPLC 96.6% (AUC), t_R = 20.88 min.

<u>Example 32</u> – Preparation of the Acetate of Cyclosporin yne-yne-ene

[0097] To an ice-cooled solution of the acetate of cyclosporin (trimethylsilyl)diyne from Example 24 (25 mg, 0.019 mmol) in triethylamine (1 mL) was added tetrabutylammonium fluoride (95 µL, 1 M in THF, 0.095 mmol), then the

mixture was stirred for 10 min. The reaction mixture was allowed to warm to room temperature, then copper(I) iodide (4 mg, 0.019 mmol) and dichlorobis(triphenylphosphine)palladium(II) (13 mg, 0.019 mmol) were added into the mixture followed by vinyl iodide (30 μL, 0.38 mmol). The resulting reaction mixture was stirred at room temperature for 1 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the desired acetate of cyclosporin yne-yne-ene (6 mg, 25%) as a brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43 (d, J = 9.5 Hz, 1H), 8.07 (d, J = 6.7 Hz, 1H), 7.82 (d, J = 8.9 Hz, 1H), 7.64 (d, J = 7.7 Hz, 1H), 5.80–5.65 (m, 4H), 5.60–5.52 (m, 3H), 5.45–5.33 (m, 2H), 5.28(dd, J = 11.9, 3.6 Hz, 1H), 5.17 (t, J = 7.7 Hz, 1H), 5.02–4.91 (m, 4H), 4.86 (t, J = 7.4 Hz, 1H), 4.79 (t, J = 9.4 Hz, 1H), 4.65 (d, J = 14.0 Hz, 1H), 4.49 (d, J = 7.0 Hz, 1H), 3.43 (s, 3H), 3.30 (s, 3H), 3.24 (s, 3H), 3.19 (s, 3H), 3.09 (s, 3H), 2.70 (s, 3H), 2.68 (s, 3H), 2.45–2.35 (m, 1H), 2.30–2.05 (m, 4H), 2.04 (s, 3H), 1.98–1.83 (m, 2H), 1.72–1.60 (m, 3H), 1.45–0.75 (m, 54H); ESI MS m/z 1279 $[C_{67}H_{111}N_{11}O_{13} + H]^+$.

<u>Example 33</u> – Preparation of Cyclosporin yne-yne-ene

[0098] To a solution of the acetate of cyclosporin yne-yne-ene from Example 32 (14 mg, 0.011 mmol) in MeOH (2 mL) was added potassium carbonate (30 mg, 0.22 mmol), then the mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with ethyl acetate (30 mL), then washed with a saturated solution of ammonium chloride (15 mL). The aqueous layer was separated and extracted with ethyl acetate (2 × 20 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford cyclosporin yne-yne-ene (10 mg, 72%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (d, J = 10.0 Hz, 1H), 7.65 (d, J = 7.5 Hz, 1H), 7.36 (d, J = 8.5 Hz, 1H), 7.20 (d, J = 8.0 Hz, 1H), 5.85–5.67 (m, 3H), 5.57 (dd, J = 10.5, 3.0 Hz, 1H), 5.46 (d, J = 7.0 Hz, 1H), 5.29 (dd, J = 11.5, 4.0 Hz, 1H), 5.12 (d, J = 10.5 Hz, 1H), 5.08 (t, J = 7.0 Hz, 1H), 5.05–4.95 (m, 2H), 4.83 (t, J = 7.0 Hz, 1H), 4.74–4.64 (m, 2H), 4.52 (t, J = 7.5 Hz, 1H), 3.93 (t, J = 6.5 Hz, 1H), 3.51 (s, 3H), 3.38 (s, 3H), 3.27 (s, 3H), 3.11 (s, 3H), 3.09 (s, 3H), 2.71 (s, 3H), 2.69 (s, 3H), 2.61 (dd, J = 17.5, 4.0 Hz, 1H), 2.45–2.35 (m, 1H), 2.26 (dd, J = 17.5, 7.5 Hz,

1H), 2.20–2.07 (m, 5H), 2.03–1.88 (m, 3H), 1.82–1.60 (m, 5H), 1.50–0.80 (m, 53H); ESI MS *m/z* 1237 [$C_{65}H_{109}N_{11}O_{12}$ + H]$^+$; HPLC >99% (AUC), t_R = 19.98 min.

<u>Example 34</u> – Preparation of the Acetate of *cis*-Cyclosporin yne-yne-ene

[0099] To an ice-cooled solution of the acetate of cyclosporin (trimethylsilyl)diyne from Example 24 (53 mg, 0.040 mmol) in triethylamine (1 mL) was added tetrabutylammonium fluoride (0.20 mL, 1 M in THF, 0.20 mmol), then the mixture was stirred for 10 min. The reaction mixture was allowed to warm to room temperature, then copper(I) iodide (4 mg, 0.02 mmol) and dichlorobis(triphenylphosphine)palladium(II) (14 mg, 0.02 mmol) were added into the mixture followed by *cis*-1-bromo-1-propene (68 μL, 0.80 mmol). The resulting reaction mixture was stirred at room temperature for 1 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the desired acetate of *cis*-cyclosporin yne-yne-ene (19 mg, 37%) as a pale-brown solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50 (d, *J* = 9.7 Hz, 1H), 8.06 (d, *J* = 6.8 Hz, 1H), 7.67 (d, *J* = 8.9 Hz, 1H), 7.58 (d, *J* = 7.8 Hz, 1H), 6.15–6.03 (m, 1H), 5.70 (dd, *J* = 11.0, 3.8 Hz, 1H), 5.58–5.40 (m, 3H), 5.31 (dd, *J* = 11.9, 3.6 Hz, 2H), 5.15 (t, *J* = 7.2 Hz, 1H), 5.04–4.82 (m, 3H), 4.74 (t, *J* = 10.5 Hz, 1H), 4.65 (d, *J* = 13.9 Hz, 1H), 4.46 (t, *J* = 7.0 Hz, 1H), 3.43 (s, 3H), 3.32 (s, 3H), 3.27 (s, 3H), 3.20 (s, 3H), 3.08 (s, 3H), 2.69 (s, 3H), 2.67 (s, 3H), 2.50–2.09 (m, 6H), 2.05 (s, 3H), 1.98–1.83 (m, 4H), 1.72–1.60 (m, 3H), 1.45–0.75 (m, 58H); ESI MS *m/z* 1293 [$C_{68}H_{113}N_{11}O_{13}$ + H]$^+$.

<u>Example 35</u> – Preparation of *cis*-Cyclosporin yne-yne-ene

[0100] To a solution of the acetate of *cis*-cyclosporin yne-yne-ene from Example 34 (19 mg, 0.015 mmol) in MeOH (2 mL) was added potassium carbonate (41 mg, 0.30 mmol), then the mixture was stirred at room temperature for 6 h. The reaction mixture was diluted with ethyl acetate (40 mL), then washed with a saturated solution of ammonium chloride (20 mL). The aqueous layer was separated and extracted with ethyl acetate (2 × 30 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford *cis*-cyclosporin yne-yne-ene (10 mg,

53%) as a white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 7.97 (d, *J* = 9.6 Hz, 1H), 7.70 (d, *J* = 7.3 Hz, 1H), 7.42 (d, *J* = 8.2 Hz, 1H), 7.25 (overlapped with $CHCl_3$, 1H), 6.15–6.04 (m, 1H), 5.71 (dd, *J* = 11.1, 3.9 Hz, 1H), 5.52–5.40 (m, 2H), 5.29 (dd, *J* = 11.5, 3.8 Hz, 1H), 5.15–4.95 (m, 5H), 4.83 (t, *J* = 7.2 Hz, 1H), 4.76–4.63 (m, 2H), 4.52 (t, *J* = 7.1 Hz, 1H), 3.91 (t, *J* = 6.4 Hz, 1H), 3.51 (s, 3H), 3.38 (s, 3H), 3.27 (s, 3H), 3.12 (s, 3H), 3.10 (s, 3H), 2.72 (s, 3H), 2.70 (s, 3H), 2.61 (dd, *J* = 17.5, 3.8 Hz, 1H), 2.50–1.85 (m, 7H), 1.80–0.78 (m, 63H); ESI MS *m/z* 1251 $[C_{66}H_{111}N_{11}O_{12} + H]^+$; HPLC >99% (AUC), t_R = 20.59 min.

<u>Example 36</u> – Preparation of the Acetate of *trans*-Cyclosporin yne-yne-ene

[0101] To an ice-cooled solution of the acetate of cyclosporin (trimethylsilyl)diyne from Example 24 (60 mg, 0.045 mmol) in triethylamine (1 mL) was added tetrabutylammonium fluoride (0.23 mL, 1 M in THF, 0.23 mmol), then the mixture was stirred for 10 min. The reaction mixture was allowed to warm to room temperature, then copper(I) iodide (4 mg, 0.02 mmol) and dichlorobis(triphenylphosphine)palladium(II) (16 mg, 0.02 mmol) were added into the mixture followed by *trans*-1-bromo-1-propene (77 μL, 0.90 mmol). The resulting reaction mixture was stirred at room temperature for 1 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the desired acetate of *trans*-cyclosporin yne-yne-ene (26 mg, 45%) as a pale-brown solid: ^1H NMR (300 MHz, $CDCl_3$) δ 8.45 (d, *J* = 9.3 Hz, 1H), 8.08 (d, *J* = 6.6 Hz, 1H), 7.82 (d, *J* = 8.7 Hz, 1H), 7.64 (d, *J* = 7.8 Hz, 1H), 6.35–6.18 (m, 1H), 5.70 (dd, *J* = 11.1, 3.9 Hz, 1H), 5.60–5.35 (m, 5H), 5.28 (dd, *J* = 12.0, 3.3 Hz, 1H), 5.17 (t, *J* = 6.3 Hz, 1H), 5.04–4.75 (m, 5H), 4.65 (d, *J* = 13.8 Hz, 1H), 4.49 (t, *J* = 7.2 Hz, 1H), 3.43 (s, 3H), 3.30 (s, 3H), 3.24 (s, 3H), 3.19 (s, 3H), 3.09 (s, 3H), 2.70 (s, 3H), 2.68 (s, 3H), 2.45–2.35 (m, 1H), 2.30–1.85 (m, 10H), 2.03 (s, 3H), 1.80 (dd, *J* = 6.9, 1.5 Hz, 3H), 1.75–1.58 (m, 3H), 1.45–0.75 (m, 52H); ESI MS *m/z* 1292 $[C_{68}H_{113}N_{11}O_{13} + H]^+$.

<u>Example 37</u> – Preparation of *trans*-Cyclosporin yne-yne-ene

[0102] To a solution of the acetate of *trans*-cyclosporin yne-yne-ene from Example 36 (25 mg, 0.019 mmol) in MeOH (3 mL) was added potassium carbonate

(52 mg, 0.38 mmol), then the mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with ethyl acetate (40 mL), then washed with a saturated solution of ammonium chloride (20 mL). The aqueous layer was separated and extracted with ethyl acetate (2 × 30 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the *trans*-cyclosporin yne-yne-ene (15 mg, 63%) as a white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, J = 9.6 Hz, 1H), 7.69 (d, J = 7.2 Hz, 1H), 7.37 (d, J = 8.4 Hz, 1H), 7.21 (d, J = 7.8 Hz, 1H), 6.35–6.20 (m, 1H), 5.71 (dd, J = 11.4, 4.2 Hz, 1H), 5.55–5.40 (m, 2H), 5.29 (dd, J = 11.4, 4.2 Hz, 1H), 5.18–4.95 (m, 4H), 4.83 (t, J = 7.2 Hz, 1H), 4.76–4.63 (m, 2H), 4.52 (t, J = 7.2 Hz, 1H), 3.91 (t, J = 6.3 Hz, 1H), 3.50 (s, 3H), 3.38 (s, 3H), 3.27 (s, 3H), 3.12 (s, 3H), 3.09 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.61 (dd, J = 17.4, 3.9 Hz, 1H), 2.45–1.85 (m, 7H), 1.80 (dd, J = 6.6, 1.5 Hz, 3H), 1.75–0.78 (m, 61H); ESI MS *m/z* 1250 $[C_{66}H_{111}N_{11}O_{12} + H]^+$; HPLC >99% (AUC), t_R = 20.28 min.

Example 38 – Preparation of Cyclosporin Alkyne

[0103] A suspension of cyclosporin alkyne from Example 3 (50 mg, 0.04 mmol), cesium carbonate (325 mg, 1.0 mmol), sodium iodide (150 mg, 1.0 mmol) and copper(I) iodide (190 mg, 1.0 mmol) in DMF (2 mL) was stirred at room temperature for 30 min. Allyl bromide (70 μL, 0.80 mmol) was added dropwise and the resulting mixture was stirred at room temperature overnight. The blue suspension was diluted with Et_2O and filtered. The filtrate was washed twice with H_2O. The combined aqueous layers were extracted with Et_2O. The combined organics were washed with brine, dried over Na_2SO_4, and concentrated. Purification by semi-preparative HPLC gave cyclosporin alkyne (25 mg, 52%) as an off-white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 8.13 (d, J = 9.5 Hz, 1H), 7.78 (d, J = 7.3 Hz, 1H), 7.48 (d, J = 8.4 Hz, 1H), 7.24 (d, J = 10.3 Hz, 1H), 5.84–5.75 (m, 1H), 5.69 (dd, J = 11.0, 4.2 Hz, 1H), 5.38–4.96 (m, 10H), 4.84 (t, J = 7.1 Hz, 1H), 4.74–4.67 (m, 2H), 4.51 (t, J = 7.3 Hz, 1H), 3.88 (t, J = 6.6 Hz, 1H), 3.49 (s, 3H), 3.37 (s, 3H), 3.27 (s, 3H), 3.14 (s, 3H), 3.09 (s, 3H), 2.93–2.70 (m, 3H), 2.72 (s, 3H), 2.70 (s, 3H), 2.15–1.99 (m, 7H), 1.90–1.57 (m, 6H), 1.43–1.28 (m, 13H), 1.06–0.82 (m, 40H); ESI MS *m/z* 1227 $[C_{64}H_{111}N_{11}O_{12} + H]^+$; HPLC >99% (AUC), t_R = 19.59 min.

Example 39 – Preparation of Cyclosporin Alkyne

[0104] A suspension of cyclosporin alkyne from Example 3 (50 mg, 0.04 mmol), cesium carbonate (326 mg, 1.0 mmol), sodium iodide (152 mg, 1.0 mmol) and copper(I) iodide (190 mg, 1.0 mmol) in DMF (2 mL) was stirred at room temperature for 30 min. Benzyl bromide (100 μL, 0.8 mmol) was added dropwise and the resulting mixture was stirred at room temperature overnight. The blue suspension was diluted with Et_2O and filtered. The filtrate was washed twice with H_2O. The combined aqueous layers were extracted with Et_2O. The combined organics were washed with brine, dried over Na_2SO_4, and concentrated. Purification by semi-preparative HPLC gave the cyclosporin alkyne (8 mg, 17%) as an off-white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 8.14 (d, J = 9.7 Hz, 1H), 7.78 (d, J = 7.3 Hz, 1H), 7.48 (d, J = 8.5 Hz, 1H), 7.35–7.30 (m, 5H), 7.21 (d, J = 7.5 Hz, 1H), 5.70 (dd, J = 11.0, 4.0 Hz, 1H), 5.38 (d, J = 6.8 Hz, 1H), 5.33–5.29 (m, 1H), 5.20–4.96 (m, 5H), 4.83 (t, J = 7.3 Hz, 1H), 4.74–4.67 (m, 2H), 4.51 (t, J = 7.2 Hz, 1H), 3.88 (t, J = 6.5 Hz, 1H), 3.57 (s, 2H), 3.52–3.45 (m, 10H), 3.38 (s, 3H), 3.25 (s, 3H), 3.13 (s, 3H), 3.09 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.16–1.62 (m, 10H), 1.43–1.19 (m, 16H), 1.01–0.79 (m, 35H); ESI MS m/z 1277 $[C_{68}H_{113}N_{11}O_{12} + H]^+$; HPLC >99% (AUC), t_R = 20.43 min.

Example 40 – Preparation of 1-(Trimethylsilyl)propyn-3-yl Cyclosporin Alkyne

[0105] A suspension of cyclosporin alkyne from Example 3 (50 mg, 0.04 mmol), cesium carbonate (326 mg, 1.0 mmol), sodium iodide (152 mg, 1.0 mmol) and copper(I) iodide (190 mg, 1.0 mmol) in DMF (2 mL) was stirred at room temperature for 30 min. 3-Bromo-1-(trimethylsilyl)-1-propyne (0.11 mL, 0.8 mmol) was added dropwise and the resulting mixture was stirred at room temperature for 15 min. The blue suspension was diluted with Et_2O and filtered. The filtrate was washed twice with H_2O. The combined aqueous layers were extracted with Et_2O. The combined organics were washed with brine, dried over Na_2SO_4, and concentrated. Purification by semi-preparative HPLC gave the 1-(trimethylsilyl)propyn-3-yl cyclosporin alkyne (26 mg, 50%) as an off-white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 8.09 (d, J = 9.4 Hz, 1H), 7.77 (d, J = 7.5 Hz, 1H), 7.47 (d, J = 8.3 Hz, 1H), 7.23 (d, J = 7.8 Hz, 1H), 5.70 (dd, J = 10.9, 3.9 Hz, 1H), 5.38 (d,

J = 6.6 Hz, 1H), 5.28 (dd, *J* = 11.5, 3.6 Hz, 1H), 5.19–4.96 (m, 4H), 4.84 (t, *J* = 7.4 Hz, 1H), 4.74–4.66 (m, 2H), 4.51 (t, *J* = 7.2 Hz, 1H), 3.83 (t, *J* = 6.6 Hz, 1H), 3.49 (s, 3H), 3.38 (s, 3H), 3.27 (s, 3H), 3.18 (s, 2H), 3.13 (s, 3H), 3.09 (s, 3H), 2.72 (s, 3H), 2.70 (s, 3H), 2.15–1.98 (m, 10H), 1.91–1.23 (m, 18H), 1.06–0.83 (m, 41H), 0.15 (s, 9H); ESI MS *m/z* 1297 [$C_{67}H_{117}N_{11}O_{12}Si + H]^+$; HPLC 96.8% (AUC), t_R = 21.59 min.

<u>Example 41</u> – Preparation of Cyclosporin Non-Conjugated Diyne

[0106] To a solution of cyclosporin alkyne from Example 40 (18 mg, 0.01 mmol) in MeOH (1 mL) was added potassium carbonate (20 mg, 0.14 mmol) and then the mixture was stirred at room temperature for 1.5 h. The mixture was diluted with EtOAc, washed with H_2O (2 ×), brine, dried over Na_2SO_4, and concentrated. Purification by semi-preparative HPLC gave the cyclosporin non-conjugated diyne (8 mg, 43%) as a white solid: ^1H NMR (500 MHz, $CDCl_3$) δ 8.10 (d, *J* = 9.7 Hz, 1H), 7.71 (d, *J* = 7.4 Hz, 1H), 7.46 (d, *J* = 8.3 Hz, 1H), 7.20 (d, *J* = 7.8 Hz, 1H), 5.71 (dd, *J* = 10.9, 4.2 Hz, 1H), 5.40 (d, *J* = 6.6 Hz, 1H), 5.28 (dd, *J* = 11.6, 3.7 Hz, 1H), 5.17 (d, *J* = 10.9 Hz, 1H), 5.09 (t, *J* = 6.7 Hz, 1H), 5.04–4.98 (m, 2H), 4.84 (app quintet, *J* = 7.2 Hz, 1H), 4.73–4.66 (m, 2H), 4.52 (app quintet, *J* = 7.3 Hz, 1H), 3.85 (t, *J* = 6.6 Hz, 1H), 3.49 (s, 3H), 3.38 (s, 3H), 3.28 (s, 3H), 3.13 (s, 3H), 3.09 (s, 3H), 2.72 (s, 3H), 2.70 (s, 3H), 2.49–2.38 (m, 2H), 2.17–2.09 (m, 5H), 2.02 (t, *J* = 2.7 Hz, 1H), 2.01–1.95 (m, 1H), 1.84–1.59 (m, 7H), 1.53–1.41 (m, 4H), 1.36–1.24 (m, 12H), 1.04–0.84 (m, 40H); ESI MS *m/z* 1225 [$C_{64}H_{109}N_{11}O_{12} + H]^+$; HPLC 99.0% (AUC), t_R = 18.61 min; and cyclosporin alkynylallene (6.7 mg, 36%) as a white solid: ^1H NMR (500 MHz, $CDCl_3$) δ 8.07 (d, *J* = 9.6 Hz, 1H), 7.75 (d, *J* = 7.2 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.29 (d, *J* = 7.8 Hz, 1H), 5.70 (dd, *J* = 10.9, 2.5 Hz, 1H), 5.40–5.35 (m, 2H), 5.29 (dd, *J* = 10.9, 4.5 Hz, 1H), 5.17 (d, *J* = 10.9 Hz, 1H), 5.10 (t, *J* = 6.4 Hz, 1H), 5.05–5.01 (m, 2H), 4.96 (d, *J* = 6.9 Hz, 1H), 4.84 (t, *J* = 7.1 Hz, 1H), 4.73–4.67 (m, 2H), 4.51 (app quintet, *J* = 7.2 Hz, 1H), 3.48 (t, *J* = 6.6 Hz, 1H), 3.38 (s, 3H), 3.26 (s, 3H), 3.14 (s, 3H), 3.10 (s, 3H), 2.72 (s, 3H), 2.70 (s, 3H), 2.15–2.09 (m, 5H), 2.02–1.61 (m, 7H), 1.55–1.25 (m, 11H), 1.04–0.84 (m, 50H); ESI MS *m/z* 1225 [$C_{64}H_{109}N_{11}O_{12} + H]^+$; HPLC 91.7% (AUC), t_R = 20.45 min.

<u>Example 42</u> – Preparation of the Acetate of Cyclosporin Non-Conjugated Diyne

[0107] A suspension of the acetate of cyclosporin alkyne from Example 6 (50 mg, 0.05 mmol), cesium carbonate (326 mg, 1.0 mmol), sodium iodide (150 mg, 1.0 mmol) and copper(I) iodide (190 mg, 1.0 mmol) in DMF (2 mL) was stirred at room temperature for 30 min. 1-Bromo-2-butyne (90 μL, 1.0 mmol) was added dropwise and the resulting mixture was stirred at room temperature overnight. The blue suspension was diluted with Et_2O and filtered. The filtrate was washed twice with H_2O. The combined aqueous layers were extracted with Et_2O. The combined organics were washed with brine, dried over Na_2SO_4, and concentrated. Purification by semi-preparative HPLC gave the acetate of cyclosporin non-conjugated diyne (47 mg, 73%) as a light brown solid: ^1H NMR (300 MHz, $CDCl_3$) δ 8.46 (d, J = 9.8 Hz, 1H), 8.10 (d, J = 6.8 Hz, 1H), 7.81 (d, J = 8.9 Hz, 1H), 7.60 (d, J = 7.7 Hz, 1H), 5.70 (dd, J = 10.9, 4.0 Hz, 1H), 5.23–5.15 (m, 6H), 5.07–4.62 (m, 5H), 4.87 (t, J = 6.9 Hz, 1H), 3.43 (s, 3H), 3.29 (s, 3H), 3.24 (s, 3H), 3.19 (s, 3H), 3.09 (s, 3H), 2.70 (s, 3H), 2.68 (s, 3H), 2.49–2.09 (m, 13H), 2.05 (s, 3H), 1.74 (t, J = 2.5 Hz, 3H), 1.41–1.26 (m, 12H), 1.12–0.82 (m, 44H); ESI MS m/z 1281 $[C_{67}H_{113}N_{11}O_{13} + H]^+$.

<u>Example 43</u> – Preparation of Cyclosporin Non-Conjugated Diyne

[0108] To a solution of the acetate of cyclosporin non-conjugated diyne from Example 42 (47 mg, 0.04 mmol) in MeOH (2 mL) was added potassium carbonate (55 mg, 0.4 mmol) and then the mixture was stirred at room temperature overnight. The mixture was diluted with EtOAc, washed with H_2O (2 ×), brine, dried over Na_2SO_4, and concentrated. Purification by semi-preparative HPLC gave the cyclosporin non-conjugated diyne (23 mg, 46%) as a brown solid: ^1H NMR (300 MHz, $CDCl_3$) δ 8.09 (d, J = 9.5 Hz, 1H), 7.77 (d, J = 7.3 Hz, 1H), 7.47 (d, J = 8.4 Hz, 1H), 7.22 (d, J = 7.9 Hz, 1H), 5.70 (dd, J = 10.9, 4.0 Hz, 1H), 5.37 (d, J = 6.7 Hz, 1H), 5.27 (dd, J = 11.5, 3.7 Hz, 1H), 5.20–4.96 (m, 5H), 4.84 (t, J = 7.3 Hz, 1H), 4.74–4.65 (m, 2H), 4.51 (t, J = 7.2 Hz, 1H), 3.85 (t, J = 6.6 Hz, 1H), 3.49 (s, 3H), 3.38 (s, 3H), 3.28 (s, 3H), 3.14 (s, 3H), 3.09 (s, 3H), 2.72 (s, 3H), 2.70 (s, 3H), 2.43–2.01 (m, 15H), 1.78 (t, J = 2.5 Hz, 3H), 1.66–1.23 (m, 15H), 1.04–0.83 (m, 40H); ESI MS m/z 1239 $[C_{65}H_{111}N_{11}O_{12} + H]^+$; HPLC >99% (AUC), t_R = 19.45 min.

<u>Example 44</u> – Preparation of Cyclosporin Alkynyl Alcohol

[0109] To a solution of cyclosporin alkyne from Example 3 (100 mg, 0.081 mmol) and paraformaldehyde (133 mg, 0.81 mmol) in DMSO (3 mL) was added benzyltrimethylammonium hydroxide (372 μL, 40% solution in methanol, 0.81 mmol) dropwise over 10 min. The resulting solution was stirred at room temperature for 14 h. The reaction was quenched with water and extracted with diethyl ether (4 × 25 mL). The combined organic layers were washed with water, brine, dried over sodium sulfate, filtered and concentrated *in vacuo*. The residue was purified by semi-preparative HPLC to afford the cyclosporin alkynyl alcohol (23 mg, 23%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (d, J = 9.3 Hz, 1H), 7.52 (d, J = 7.3 Hz, 1H), 7.46 (d, J = 8.3 Hz, 1H), 7.20 (d, J = 7.9 Hz, 1H), 5.69 (dd, J = 10.8, 4.3 Hz, 1H), 5.45–5.40 (m, 2H), 5.14 (d, J = 11.0 Hz, 1H), 5.13–5.05 (m, 2H), 5.00–4.95 (m, 2H), 4.83 (t, J = 6.8 Hz, 1H), 4.64 (dd, J = 9.8, 8.4 Hz, 1H), 4.52 (t, J = 7.3 Hz, 1H), 4.03 (d, J = 6.7 Hz, 2H), 3.94 (t, J = 6.7 Hz, 1H), 3.51 (s, 3H), 3.31 (s, 3H), 3.27 (s, 3H), 3.13 (s, 3H), 3.09 (s, 3H), 2.70 (s, 3H), 2.69 (s, 3H), 2.40–0.70 (m, 70H); ESI MS *m/z* 1217 $[C_{62}H_{109}N_{11}O_{13} + H]^+$; HPLC 98.4% (AUC), t_R = 18.24 min.

<u>Example 45</u> – Preparation of Cyclosporin Diol

[0110] To a mechanically stirred solution of diisopropylamine (2.6 mL, 18 mmol) in THF (50 mL) at –78°C was added dropwise *n*-butyllithium (6.6 mL, 2.5 M in hexane, 17 mmol), then the mixture was stirred for 0.5 h. A solution of cyclosporin A (1.0 g, 0.83 mmol) in THF (8 mL) was added, and then the mixture was stirred for 2 h at –78°C. Paraformaldehyde (8.0 g) was heated to 170°C and the resulting formaldehyde gas was transferred into the reaction via a glass tube which was wrapped with cotton and aluminum foil over 2 h. After stirring another 1 h at -78°C, the reaction mixture was quenched with water (10 mL). The mixture was allowed to warm to room temperature, diluted with ethyl acetate (150 mL), and washed with water (2 × 50 mL). The organic layer was separated, dried over anhydrous sodium sulfate, and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford cyclosporin diol (0.45 g, 44%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (d, J = 9.9 Hz, 1H), 7.70 (d, J = 7.4 Hz, 1H), 7.57 (d, J = 8.2 Hz, 1H), 7.15 (overlapped with $CHCl_3$, 1H), 5.70 (dd, J

= 11.0, 4.0 Hz, 1H), 5.49 (d, J = 6.4 Hz, 1H), 5.38–5.30 (m, 3H), 5.16–4.93 (m, 5H), 4.83 (t, J = 7.2 Hz, 1H), 4.65 (t, J = 9.5 Hz, 1H), 4.54 (t, J = 7.2 Hz, 1H), 4.05 (d, J = 6.8 Hz, 2H), 3.73 (t, J = 6.3 Hz, 1H), 3.49 (s, 3H), 3.30 (s, 3H), 3.25 (s, 3H), 3.15 (s, 3H), 3.11 (s, 3H), 2.70 (s, 3H), 2.69 (s, 3H), 2.50–2.38 (m, 2H), 2.20–1.92 (m, 6H), 1.75–0.65 (m, 64H); ESI MS m/z 1233 $[C_{63}H_{113}N_{11}O_{13} + H]^+$.

<u>Example 46</u> – Preparation of Cyclosporin Diacetate

[0111] To a solution of cyclosporin diol from Example 45 (0.43 g, 0.35 mmol) in methylene chloride (5 mL) was added pyridine (0.57 mL, 7.0 mmol) followed by 4-(dimethylamino)pyridine (86 mg, 0.70 mmol) and acetic anhydride (1.0 mL, 10.5 mmol). The reaction mixture was stirred for 2 days at room temperature. The reaction was diluted with ethyl ether (150 mL) and washed with a saturated solution of sodium bicarbonate (30 mL), 1N HCl solution (30 mL) and brine (30 mL). The organic layer was separated, dried over anhydrous sodium sulfate, and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford cyclosporin diacetate (0.23 g, 50%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.60 (d, J = 9.8 Hz, 1H), 8.05 (d, J = 6.6 Hz, 1H), 7.55 (d, J = 7.8 Hz, 1H), 7.49 (d, J = 9.3 Hz, 1H), 5.68 (dd, J = 11.0, 4.0 Hz, 1H), 5.49 (s, 2H), 5.40–4.95 (m, 8H), 4.85 (t, J = 7.5 Hz, 1H), 4.76 (t, J = 9.3 Hz, 1H), 4.58–4.34 (m, 3H), 3.37 (s, 3H), 3.27 (s, 3H), 3.23 (s, 3H), 3.20 (s, 3H), 3.14 (s, 3H), 2.67 (s, 3H), 2.66 (s, 3H), 2.48–2.35 (m, 1H), 2.10 (s, 3H), 2.01 (s, 3H), 1.98–1.85 (m, 2H), 1.75–0.65 (m, 67H); ESI MS m/z 1317 $[C_{67}H_{117}N_{11}O_{15} + H]^+$.

<u>Example 47</u> – Preparation of Cyclosporin Aldehyde

[0112] Ozone was bubbled into a solution of cyclosporin diacetate from Example 46 (0.22 g, 0.17 mmol) in methylene chloride (10 mL) at –78°C until a blue color was developed. The mixture was degassed with nitrogen for a few minutes and dimethylsulfide (0.4 mL) was added at –78°C. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. The reaction mixture was concentrated in vacuo and the residue was dissolved in ethyl acetate (120 mL), washed with water (2 × 20 mL) and brine (30 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford cyclosporin aldehyde (0.19 g, 86%) as a white solid. The crude

material was carried to the next step without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.55 (d, J = 3.4 Hz, 1H), 8.60 (d, J = 9.9 Hz, 1H), 7.96 (d, J = 7.1 Hz, 1H), 7.53 (d, J = 7.7 Hz, 1H), 7.33 (d, J = 9.1 Hz, 1H), 5.68 (dd, J = 11.0, 4.0 Hz, 1H), 5.53 (d, J = 11.2 Hz, 1H), 5.47 (d, J = 11.2 Hz, 1H), 5.30 (dd, J = 12.3, 3.6 Hz, 1H), 5.18–4.92 (m, 5H), 4.84 (t, J = 6.9 Hz, 1H), 4.72 (t, J = 9.6 Hz, 1H), 4.55–4.35 (m, 3H), 3.39 (s, 3H), 3.30 (s, 3H), 3.29 (s, 3H), 3.21 (s, 3H), 3.12 (s, 3H), 2.66 (s, 3H), 2.65 (s, 3H), 2.48–2.30 (m, 3H), 2.10 (s, 3H), 1.99 (s, 3H), 1.80–0.75 (m, 64H); ESI MS *m/z* 1305 $[C_{65}H_{113}N_{11}O_{16} + H]^+$.

Example 48 – **Preparation of Cyclosporin Alkyne**

[0113] To a solution of cyclosporin aldehyde from Example 47 (715 mg, 0.55 mmol) in methanol (7.5 mL) was added potassium carbonate (760 mg, 5.5 mmol) followed by a solution of dimethyl (1-diazo-2-oxopropyl)phosphonate (1.06 g, 5.5 mmol) in methanol (4.5 mL). The resulting mixture was stirred at room temperature overnight. The solution was concentrated under reduced pressure, and then diluted with ethyl acetate (100 mL). The organic layer was washed with water (40 mL). The aqueous layer was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over anhydrous sodium sulfate, then concentrated under reduced pressure. The crude material was purified by semi-preparative HPLC to yield cyclosporin alkyne (106 mg, 16%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (d, J = 9.9 Hz, 1H), 7.62–7.55 (m, 2H), 7.27 (d, J = 9.6 Hz, 1H), 5.68 (dd, J = 11.0, 3.8 Hz, 1H), 5.47–5.41 (m, 2H), 5.18–4.92 (m, 6H), 4.91–4.77 (m, 2H), 4.63 (t, J = 9.1 Hz, 1H), 4.52 (t, J = 7.1 Hz, 1H), 4.03 (d, J = 6.6 Hz, 1H), 3.50 (s, 3H), 3.30 (s, 3H), 3.28 (s, 3H), 3.14 (s, 3H), 3.09 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.50–2.21 (m, 3H), 2.20–1.57 (m, 16H), 1.56–0.72 (m, 54H); ESI MS *m/z* 1217 $[C_{62}H_{109}N_{11}O_{13} + H]^+$; HPLC >99% (AUC), t_R = 18.20 min.

Example 49 – **Preparation of Cyclosporin yne-ene**

[0114] To a solution of cyclosporin alkyne from Example 48 (43 mg, 0.04 mmol) in triethylamine (1.5 mL) was added copper(I) iodide (4 mg, 0.02 mmol), followed by dichlorobis(triphenylphosphine)palladium(II) (14 mg, 0.02 mmol) and then vinyl iodide (123 mg, 0.8 mmol). The resulting mixture was stirred at room

temperature for 2 h. The solution was filtered through a micro filter and concentrated under reduced pressure. The crude material was purified by semi-preparative HPLC to yield cyclosporin yne-ene (106 mg, 16%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (d, J = 10.3 Hz, 1H), 7.75 (d, J = 7.0 Hz, 1H), 7.48 (d, J = 8.2 Hz, 1H), 7.26 (overlapped with $CHCl_3$, 1H), 5.81–5.66 (m, 2H), 5.59–5.50 (m, 1H), 5.46–5.33 (m, 2H), 5.26 (dd, J = 11.6, 3.7 Hz, 1H), 5.20–5.01 (m, 5H), 4.95 (t, J = 6.9 Hz, 1H), 4.84 (t, J = 7.6 Hz, 1H), 4.69 (t, J = 9.2 Hz, 1H), 4.52 (t, J = 7.5 Hz, 1H), 4.03 (d, J = 6.5 Hz, 2H), 3.84 (t, J = 6.4 Hz, 1H), 3.50 (s, 3H), 3.31 (s, 3H), 3.29 (s, 3H), 3.14 (s, 3H), 2.70 (s, 3H), 2.69 (s, 3H), 2.61–2.50 (m, 1H), 2.22–1.54 (m, 16H), 1.53–0.70 (m, 54H); ESI MS m/z 1243 $[C_{64}H_{111}N_{11}O_{13} + H]^+$; HPLC 96.3% (AUC), t_R = 21.22 min.

<u>Example 50</u> - Concanavalin A- Stimulated Splenocyte Assay

[0115] Male BALB/c mice, at 5 to 7 weeks of age, were sacrificed by CO_2 inhalation. Spleens were removed and dissociated by pushing through a nylon cell strainer. The splenocytes were washed in RPMI 1640/5% fetal calf serum (FCS) and pelleted at 400×g. Red blood cells were then lysed by resuspending the cell pellet in ACK lysis buffer (150 mM NH_4Cl, 1 mM $KHCO_3$, 0.1 mM EDTA, 3 mL per spleen) for 10 min at room temperature. After pelleting at 400×g, the cells were washed by resuspending in RPMI 1640/5% FCS and repelleting. The cell pellet was resuspended in RPMI 1640/5% FCS and again passed through a cell strainer to remove cell aggregates. The cells were then counted and adjusted to 2×10^6 cells/ml in RPMI 1640/10% FCS/50 μM 2-mercaptoethanol. Cell viability was assessed by Trypan blue staining. Cyclosporin A or the test compound and two micrograms of concanavalin A were added to the wells of a 96 well plate, prior to the addition of 2×10^5 splenocytes. The cells were cultured in a 37°C CO_2 incubator for 2 days and then pulsed with 1 μCi of [^{3}H]thymidine for 6 hours. Cells were harvested onto filtermats with a TomTec 96 well plate harvester and lysed with H_2O. The filtermat and scintillation fluid were sealed in a plastic sleeve. [^{3}H]thymidine incorporation was measured with a Wallac Trilux plate counter. Initial screens were done at a fixed value of 100 ng/ml test compound. IC_{50}s were calculated from 7 point concentration-response curves using graphPad software.

<u>Example 51</u> - Murine *Ex Vivo* Pharmacodynamic Assay

[0116] *In vivo* immunosuppressive activity can be determined for cyclosporin A and the disclosed cyclosporin analog compounds, as described below. The concanavalin A-stimulated splenocyte activity can be assessed *in vivo* using a method previously described by Peterson et al. (Peterson et al., "A Tacrolimus-Related Immunosuppressant with Biochemical Properties Distinct from Those of Tacrolimus," *Transplantation*, 65:10-18 (1998), which is hereby incorporated by reference in its entirety) or a slightly modified version thereof.

[0117] Optimal doses of cyclosporin A or an immunosuppressive compound of the present invention (four different doses of test drug plus a control set of animals with no drug) were administered orally or intravenously to male BALB/c or female C57BL mice. Three mice were tested at each dose. Concanavalin A was injected into the tail vein of the mouse at 4 hours after the administration of cyclosporin A or the immunosuppressive compound. One hour after the concanavalin A injection, the mice were euthanized, the spleens were removed under sterile conditions, and the extent of splenocyte proliferation was measured in a similar manner, as described in Example 50. The percent inhibition relative to control was plotted graphically versus the dose of the immunosuppressive compound and an ED_{50} value was determined. Each dose-response assay for the compound of the present invention was accompanied by a cyclosporin control at a single dose equal to the ED_{50}.

<u>Example 52</u> - Assay for Inhibition of Peptidyl Prolyl Isomerase Activity of Cyclophilin A

[0118] The assay for inhibition of peptidyl prolyl isomerase activity of cyclophilin A is a modification of the procedure described by Kofron et al., "Determination of Kinetic Constants for Peptidyl Prolyl *cis-trans* Isomerases by an Improved Spectrophotometric Assay," *Biochemistry* 30:6127-6134 (1991), which is hereby incorporated by reference in its entirety. Recombinant human cyclophilin A in 50 mM HEPES, 100 mM NaCl pH 8.0 is precooled to 4°C. Test compounds and the cyclosporin positive control are dissolved in dimethyl sulfoxide (DMSO) and introduced over a range of concentrations. Chymotrypsin is then added to a final concentration of 6 mg/ml. The peptide substrate, Suc-Ala-Ala-Pro-Phe-pNA (SEQ ID NO:1), is dissolved in 470 mM LiCl in trifluoroethanol and then added to 25 μg/ml to

initiate the reaction. After rapid mixing, the absorbance at 390 nm is monitored over a 90 second time course.

<u>Example 53</u> - Cellular Assay for Determination of HIV Inhibition

[0119] The *in vitro* anti-HIV activity of compounds of the present invention is measured in established cell line cultures as described by Mayaux et al., "Triterpene Derivatives That Block Entry of Human Immunodeficiency Virus Type 1 Into Cells," *Proc. Natl. Acad. Sci. USA* 91:3564-3568 (1994), which is hereby incorporated by reference in its entirety. The CEM4 cell line was infected with HIV-1_{Lai} strain. The inhibition of HIV replication in the culture is estimated by the measure of the reverse transcriptase (RT) produced in the supernatant. Anti-viral activity is expressed as the IC_{50} RT, the concentration required to reduce replication of HIV by 50%, and is determined by linear regression.

<u>Example 54</u> – Intracellular Replication of the HCV Genome *in vitro*

[0120] The effect of the cyclosporin compounds of the present invention on the intracellular replication of the HCV genome *in vitro*, using an HCV replicon system in a cultured human hepatoma Huh7 cell line is determined by the method of Lohmann et al., "Replication of Subgenomic Hepatitis C Virus RNAs in a Hepatoma Cell Line," *Science* 285:110-113 (1999), which is hereby incorporated by reference in its entirety.

<u>Example 55</u> –*In vitro* HCV Infection Experiment

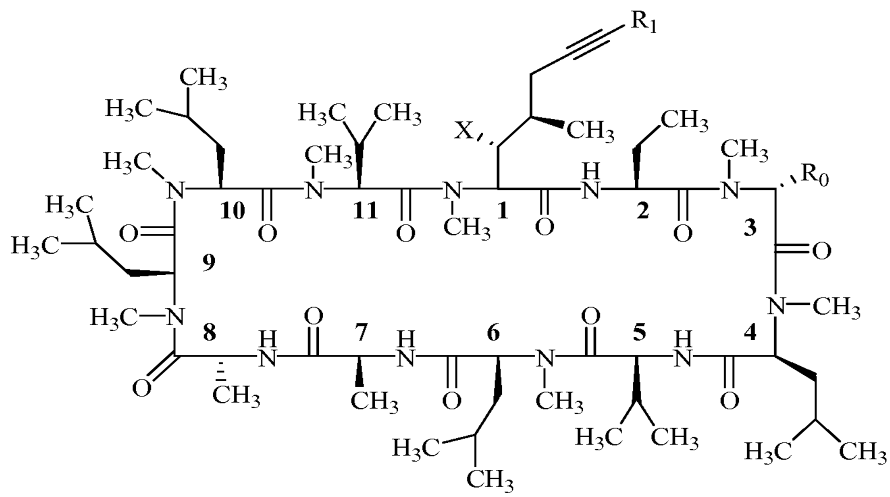
[0121] The *in vitro* HCV infection experiment is performed as described by Kato et al., "Replication of Hepatitis C Virus in Cultured Non-Neoplastic Human Hepatocytes," *Jpn. J. Cancer Res.* 87:787-792 (1996), which is hereby incorporated by reference in its entirety, and Ikada et al., "Human Hepatocyte Clonal Cell Lines That Support Persistent Replication of Hepatitis C Virus," *Virus Res.* 56:157-167 (1998), which is hereby incorporated by reference in its entirety.

[0122] Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

WHAT IS CLAIMED IS:

1. A method of preventing or treating a mammal with a viral-induced disorder comprising:

administering to the mammal a therapeutically effective amount of a compound having the following formula:



Formula I

wherein:

X is OH or OAc;

R_0 is H, CH_2OH, or CH_2OR_2;

R_1 is selected from the group consisting of:

hydrogen;

halogen;

C_2-C_6 saturated or unsaturated, straight or branched carbon chain;

C_2-C_6 saturated or unsaturated, straight or branched carbon chain containing substitution or substitutions selected from the group consisting of deuterium, halogen, nitrogen, sulfur, and silicon atom or atoms;

C_2-C_6 saturated or unsaturated, straight or branched carbon chain containing a function group or function groups selected from the group consisting of alcohol, ether, aldehyde, ketone, carboxylic ester, and amide;

C_2-C_4 saturated or unsaturated, straight or branched carbon chain containing an aryl or a heteroaryl;

C_3-C_6-substituted and unsubstituted cycloalkyl;

substituted and unsubstituted aryl;

substituted and unsubstituted heteroaryl;

-CH_2OH;
-CHO;
-CH=N-OR_3; and
-CH=N-NR_3R_4;

R_2 is selected from the group consisting of:
alkanoyl;
alkenoyl;
alkynoyl;
aryloyl;
arylalkanoyl;
alkylaminocarbonyl;
arylaminocarbonyl;
arylalkylaminocarbonyl;
alkyloxycarbonyl;
aryloxycarbonyl; and
arylalkyloxycarbonyl;

R_3 or R_4 are the same or different and independently selected from the group consisting of:
hydrogen;
C_1-C_6 saturated straight or branched carbon chain;
C_3-C_6 unsaturated straight or branched carbon chain;
C_3-C_6-substituted and unsubstituted cycloalkyl;
C_1-C_4 carbon chain containing an aryl or heteroaryl;
substituted and unsubstituted aryl;
substituted and unsubstituted heteroaryl;
alkanoyl;
alkenoyl;
alkynoyl;
aryloyl;
arylalkanoyl;
alkylaminocarbonyl;
arylaminocarbonyl;
arylalkylaminocarbonyl;
alkyloxycarbonyl;
aryloxycarbonyl; and
arylalkyloxycarbonyl; and

R_3 together with R_4 results in the formation of a cyclic moiety of C_2-C_6 optionally containing heteroatom or heteroatoms,

or a pharmaceutically acceptable salt thereof,

under conditions effective to prevent or treat the viral-induced disorder.

2. The method according to claim 1, wherein X is OH or OAc, and R_0 is H, CH_2OH, or CH_2OAc.

3. The method according to claim 2, wherein R_1 is H.

4. The method according to claim 2, wherein R_1 is selected from the group consisting of F, Cl, Br, and I.

5. The method according to claim 2, wherein R_1 is selected from the group consisting of $CH=CH_2$, $CH=CHCH_3$, $CH=CHCH_2CH_3$, $C(CH_3)=CH_2$, $CH=CD_2$, $CH=CHCD_3$, and $CH=CDCD_3$, and wherein the carbon-carbon double bond is a cis or a trans geometric isomer or a mixture of both cis and trans geometric isomers.

6. The method according to claim 2, wherein R_1 is selected from the group consisting of $CH=CHF$, $CH=CHCl$, $CH=CHBr$, $CH=CHI$, $CH=CF_2$, and $CH=CCl_2$, and wherein the carbon-carbon double bond is a cis or a trans geometric isomer or a mixture of both cis and trans geometric isomers.

7. The method according to claim 2, wherein R_1 is selected from the group consisting of $C\equiv CH$, $C\equiv CCH_3$, $C\equiv CCD_3$, $C\equiv CCH_2CH_3$, $C\equiv CCH_2CH_2CH_3$, and $C\equiv C$-cyclopropyl.

8. The method according to claim 2, wherein R_1 is selected from the group consisting of $CH_2C\equiv CH$, $CH_2C\equiv CCH_3$, $CH_2C\equiv CCH_2CH_3$, $CH_2CH=CH_2$, $CH_2CH=CHCH_3$, and $CH_2CH=CHCH_2CH_3$, and wherein the carbon-carbon double bond is a cis or a trans geometric isomer or a mixture of both cis and trans geometric isomers.

9. The method according to claim 2, wherein R_1 is selected from the group consisting of $C\equiv C\text{-}C\equiv CH$, $C\equiv C\text{-}C\equiv CCH_3$, $C\equiv CCH=CH_2$, $C\equiv CCH=CHCH_3$, $CH=CHC\equiv CH$, $CH=CHC\equiv CCH_3$, $CH=CHCH=CH_2$, and $CH=CHCH=CHCH_3$, and

wherein the carbon-carbon double bond is a cis or a trans geometric isomer or a mixture of both cis and trans geometric isomers.

10. The method according to claim 2, wherein R_1 is cyclopropyl.

11. The method according to claim 2, wherein R_1 is selected from the group consisting of CH_2OH, -CHO, $CH(OH)CH_3$, $C(=O)CH_3$, $CH=N-OCH_3$, $CH=N-OCH_2CH_3$, $CH=N-NHCH_3$, and $CH=N-N(CH_3)_2$.

12. The method according to claim 1, wherein the viral-induced disorder is a human immunodeficiency virus-induced disorder.

13. The method according to claim 12, wherein said compound is administered in combination with antiretroviral agents selected from the group consisting of nucleoside reverse transcriptase inhibitors, nonnucleoside reverse transcriptase inhibitors, human immunodeficiency virus protease inhibitors, fusion inhibitors, and combinations thereof.

14. The method according to claim 13, wherein the nucleoside reverse transcriptase inhibitor is selected from the group consisting of Zidovudine, Didanosine, Stavudine, and Lamivudine.

15. The method according to claim 13, wherein the nonnucleoside reverse transcriptase inhibitor is selected from the group consisting of Nevirapine, Efavirenz, and Delavirdine.

16. The method according to claim 13, wherein the human immunodeficiency virus protease inhibitor is selected from the group consisting of Saquinovir, Indinavir, and Ritonavir.

17. The method according to claim 13, wherein the fusion inhibitor is Enfuvirtide.

18. The method according to claim 1, wherein the viral-induced disorder is a hepatitis C virus-induced disorder.

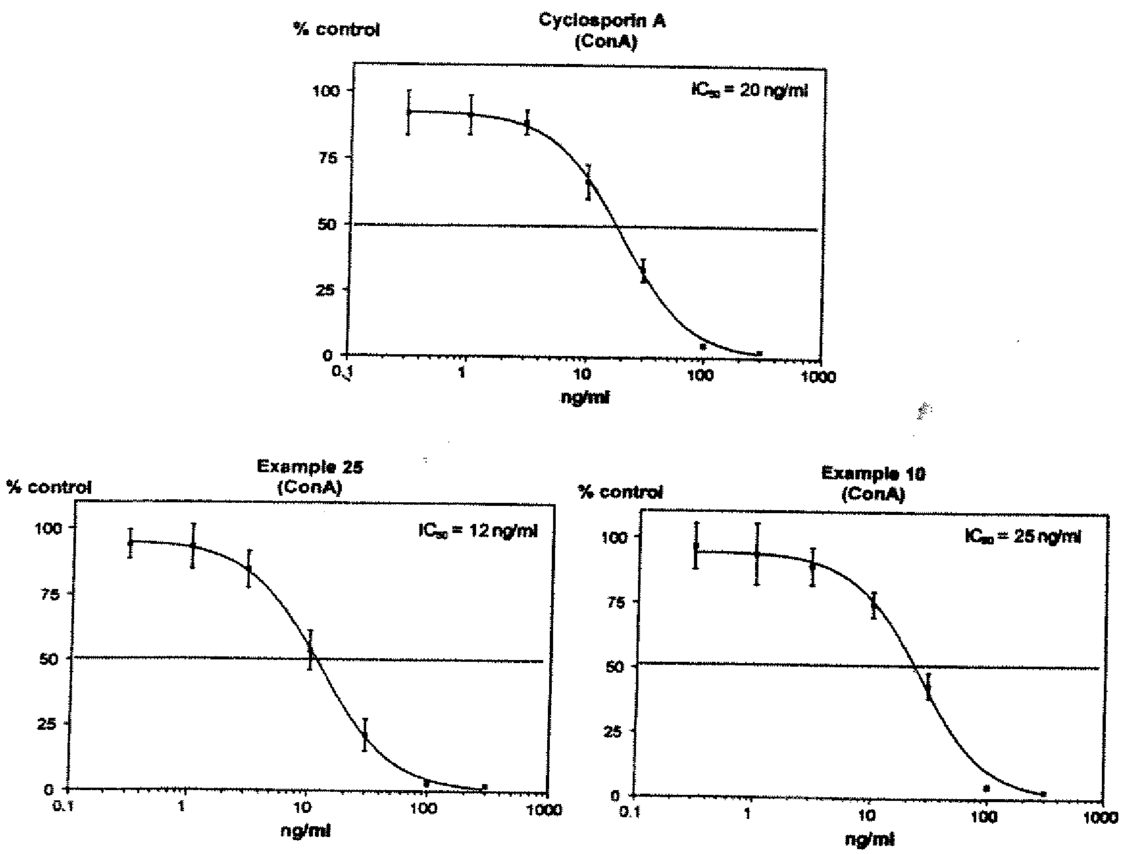
19. The method according to claim 18, wherein said compound is administered in combination with an interferon.

20. The method according to claim 19, wherein the interferon is interferon $\alpha 2a$ or interferon $\alpha 2b$.

21. The method according to claim 19, wherein the interferon is a pegylated interferon.

22. The method according to claim 21, wherein the pegylated interferon is pegylated interferon $\alpha 2a$ or pegylated interferon $\alpha 2b$.

Figure 1


% control
Cyclosporin A
(ConA)
IC50 = 20 ng/ml
100
75
50
25
0
0.1
1
10
100
1000
ng/ml
Example 25
(ConA)
% control
IC50 = 12 ng/ml
ng/ml
Example 10
(ConA)
% control
IC50 = 25 ng/ml
ng/ml